PROCEEDINGS

OF THE

NATIONAL ACADEMY OF SCIENCES

INDIA

1960

Vol. XXX SECTION - B Part IV

NATIONAL ACADEMY OF SCIENCES, INDIA
ALLAHABAD

THE NATIONAL ACADEMY OF SCIENCES, INDIA

(Registered under Act XXI of 1860)

Founded 1930

Council for 1960

President

Dr. M. S. Randhawa, M.Sc., D.Sc., F.N.I., F.N.A.Sc., I.C.S., New Delhi

Vice-Presidents

Prof. K. Banerjee, D.Sc., F.N.I., F.N.A.Sc., Calcutta
Prof. P. S. Gill, M.S., Ph.D., F.A.P.S., F.N.I., F.N.A.Sc., Aligarh

Honorary Treasurer

Prof. S. Ghosh, D.Sc., F.R.I.C., F.N.I., F.N.A.Sc., Allahabad

Foreign Secretary

Prof. B. N. Prasad, Ph.D., D.Sc., F.N.I., F.N.A.Sc., Allahabad

General Secretaries

Dr. R. N. Tandon, M.Sc., Ph.D., D.I.C., F.A.Sc., F.N.A.Sc., Allahabad
Dr. K. N. Mathur, D.Sc., F.Inst.P., F.N.A.Sc., New Delhi

Members

Prof. N. R. Dhar, D.Sc., F.R.I.C., F.N.I., F.N.A.Sc., Allahabad
Prof. A. K. Bhattacharya, D.Sc., F.R.I.C., F.N.A.Sc., Sagar
Prof. S. Ranjan, M.Sc., D.Sc., F.N.I., F.N.A.Sc., Allahabad
Dr. K. S. Krishnan, D.Sc., F.R.S., F.N.I., F.N.A.Sc., New Delhi
Prof. P. L. Srivastava, M.A., D.Phil., F.N.I., F.N.A.Sc., Allahabad
Dr. L. A. Ramdas, M.A., Ph.D., F.N I., F.N.A.Sc., New Delhi
Prof. M. D. L. Srivastava, D.Sc., F.N.A.Sc., Allahabad
Dr. S. H. Zaheer, M.A., Dr. Phil. Nat., F.N.A.Sc., Hyderabad-Dn.
Dr. A. C. Joshi, D Sc., F.N.I., F.N.A.Sc., Solan

The *Proceedings of the National Academy of Sciences, India,* is published in two sections: Section A (Physical Sciences) and Section B (Biological Sciences). Four parts of each section are published annually (since 1960).

The Editorial Board in its work of examining papers received for publication is assisted, in an honorary capacity, by a large number of distinguished scientists. Papers are accepted from members of the Academy *in good standing.* In case of a joint paper, one of the authors must be a member of the Academy. The Academy assumes no responsibility for the statements and opinions advanced by the authors. The papers must conform strictly to the rules for publication of papers in the *Proceedings.* A total of 50 reprints are supplied free of cost to the author or authors. The authors may have any reasonable number of additional reprints at cost price, provided they give prior intimation while returning the proof.

Communications regarding contributions for publication in the *Proceedings,* books for review, subscriptions etc., should be sent to the General Secretary, National Academy of Sciences, India, Lajpatrai Road, Allahabad-2 (India).

Annual Subscription for both Sections : Rs. 65; for each Section : Rs. 35 (including Annual Number); Single Copy : Rs. 7.50; Annual Number Rs. 5

PROCEEDINGS

OF THE

NATIONAL ACADEMY OF SCIENCES

INDIA

1960

VOL. XXX SECTION - B PART IV

BLACKENING OF BAJRA (*PENNISETUM TYPHOIDES* STAPF AND HUBBARD) GRAINS IN EAR HEADS CAUSED BY *CURVULARIA LUNATA* (WAKK) BOED. SYN. *C. PENNISETI* (MITRA) BOED

By

R. L. MATHUR, B. L. MATHUR *and* G. C. BHATNAGAR

Plant Pathology Section, Department of Agriculture, Rajasthan, Udaipur

[Received on 25th May, 1960]

Mitra (1921) reported leaf spot disease of bajra due to *Acrothecium penniseti* Mitra later renamed as *Curvularia penniseti* (Mitra) Boed. Rao and Salam (1954) described blackening of bajra grains on ear heads due to *C. penniseti*. Blackening of individual bajra grains in the ear heads often mistaken for smut sori, was observed during the wet months of August and September in Rajasthan. This was found to be due to brown to black mouldy growth which resulted in shrivelling of grain and reduction in their size and weight. In some cases, browning of glumes and mouldy growth on discoloured lower leaves was also observed.

The examination of a large number of diseased grains from the ears and the isolations made therefrom, yielded the fungus *Curvularia lunata* (Wakk) Boed. in vast majority and occasionally *C. maculans*, *C. uncinata* and small spored species of *Helminthosporium*. Since *C. lunata* was the most predominant and had not been reported on *Pennisetum typhoides* so far from any country, detailed studies on the morphology, pathogenicity, physiology and taxonomy of the fungus were undertaken in this laboratory and compared with *C. penniseti*. The results are reported in the present paper.

MORPHOLOGY OF THE FUNGUS

The fungus produces olive brown to olive green raised colonies with olive brown substrate colour and profuse mycelial growth on culture media such as Oat meal agar, Corn meal agar, Potato dextrose agar, Brown's medium, Sabouraud's medium and Smith Humfeld medium.

The conidiophores are simple, septate, brown, straight or bent, geniculate at the tip, measuring 64—240 $\mu \times 3{\cdot}2$—4·8 μ. The conidia are mostly three septate, brown, curved or straight with third cell from the base broader and darker than the others and basal cell having a scar showing the point of attachment to the conidiophore. The hilum is included within the contour of the rounded basal cell. The walls of the conidia are thin, measuring less than 0·5 μ in thickness. The size of conidia varied on different media and the measurements are given in table No. 1.

TABLE 1

Size of conidia of *C. lunata* (Wakk) Boed. on different media
(Count of 50 conidia)

Medium	Length in μ		Width in μ	
	Range	Mean	Range	Mean
1. Oat meal agar	16·2—28·8	23·0	7·2—10·8	9·0
2. Potato dextrose agar	12·6—18·0	14·1	9·0—10·8	9·5
3. Corn meal agar	18·0—25·2	22·1	7·2—10·8	9·9
4. Brown's agar	21·6—25·2	24·1	7·2—10·8	10·0
5. Sabouraud's medium	16·2—25·2	21·7	5·4—10·8	9·5
6. Smith Humfeld medium	19·8—27·0	23·6	9·0—12·0	10·5

The characters of the fungus are in close agreement with those of *Curvularia lunata* (Wakk) Boed.

PATHOGENICITY TESTS

Infection experiment was carried out by spraying spore suspension from the pure culture of the fungus on Bajra ear heads at different stages of grain formation. After inoculation, the ears were covered with alkathene bags and high humidity was maintained by spraying water daily. The earliest symptoms of disease evidenced by the production of light brown spots were observed on mature grains after three days of inoculation and after seven days, the infection had well advanced. The control plants and the infected plants in which grain formation had just started, remained healthy, showing thereby that the fungus could infect only mature grains. A close similarity was observed in the symptoms produced on the natural

and artificially infected grains and the latter yielded the typical fungus on reisolation.

Seedlings raised from surface sterilized seeds were also sprayed with spore suspension twice at interval of three days and placed in moist chambers. No mould symptoms appeared even after one week.

PHYSIOLOGICAL STUDIES OF THE FUNGUS

(1) *Temperature*:

To determine the cardinal temperature for the growth of the fungus, Oat meal agar plates were inoculated with 2 mm. disc of mycelium from the periphery of 4 days old culture and incubated for five days at different temperatures. The radial growth was determined by averaging two diameters of each colony and the results are represented in table No. 2.

TABLE 2

Growth of *C. lunata* at different temperatures

Temperature in Centigrade	Colony diameter (mm.)
5°	22
26°	64
29°	74
32·5°	63
35°	54
37°	52

The optimum temperature for the growth of the fungus was found to be 28°C, the maximum being 40°C.

(*ii*) *Hydrogen-ion concentration* :

The fungus was grown on potato dextrose agar adjusted to different pH values with N/20 NaOH or Acetic acid. The inoculation method was the same as that used in the temperature studies. The average diameter of each colony was recorded after incubation for seven days at 28°C and the results are presented in Table No. 3.

TABLE 3

Growth of *C. lunata* at different Hydrogen-ion concentrations

Hydrogen-ion concentrations	Colony diameter (mm.)
4·5	55
5	58·5
5·5	67·5
6·5	85
7	74·5
8	68
9	58·5

The optimum pH for the growth of the fungus was 6·5, although growth occurred at all other pH values also.

(iii) Utilization of nitrogenous substances :

The fungus, when grown on Richard's medium (KNO_3 – 10 gms; KH_2PO_4 – 5 gms; $MgSO_4$ – 2·5 gms ; $FeCl_3$ – 0·2 gms; Sucrose – 50 gms; Distilled water - 1,000 c.c.) to which different nitrogenous substances were added in equivalent proportions in place of Potassium nitrate, could utilize organic and inorganic substances except the inorganic salts of ammonia as shown in Table No. 4

TABLE 4

Growth of *C. lunata* on modified Richard's agar medium containing various nitrogen compounds.

Source of nitrogen	Colony characters	Colony diameter after seven days in mm.	Sporulation
1. Asparagin	Colony raised olive green in centre, outer ring white, dark olive brown substrate	62	Heavy
2. Potassium nitrate	Aerial mycelium slightly raised olive brown, substrate dark brown	51	,,
3. Sodium nitrate	do.	45	Moderate
4. Ammonium tartarate	Mycelium brown, substrate light brown	51	,,
5. Ammonium phosphate	White mycelium, slightly raised	10	Poor
6. Ammonium sulphate	Scanty growth	4	No
7. Ammonium nitrate	do.	5	,,

Asparagin proved to be the best source of nitrogen for growth and sporulation of the fungus.

(iv) Utilization of carbon compounds :

The fungus, when grown on Richard's medium (minus sucrose) to which different carbon compounds were added in equivalent proportions, showed that glucose and sorbitol were the best sources for its growth. Mannitol, maltose, sucrose, lactose and fructose were also utilized fairly well. Glucose, sucrose and maltose supported heavy sporulation, as shown in Table No. 5.

TABLE 5

Growth of *C. lunata* on modified Richard's agar medium containing various corbon compounds

Source of carbon	Colony charcters	Colony diameter after 7 days in mm.	Sporulation
1. Sucrose	Aerial mycelium slightly raised, olive brown, substrate dark brown	49	Heavy
2. Maltose	Colony whitish olive green, substrate dark green	46	,,
3. Glucose	Central olive green, outer orange, substrate dark olive green	60	,,
4. Sorbitol	Mycelium thin, central olive green, outer ring white	60	Moderate
5. Lactose	Mycelium olive green, slightly raised, substrate dark olive green	48	,,
6. Mannitol	Aereial mycelium thin, central olive green, outer portion white, substrate dark olive brown	44	,,
7. Fructose	Central olive green, outer portion raised, substrate dark olive green	37	Slight

TAXONOMY OF THE FUNGI CAUSING BLACK MOULD OF BAJRA

Mitra (1921) and Rao and Salam (1954) attributed the black leaf spot and sooty grains of Bajra to *C. penniseti*, but in the present studies, the causal organism was found to be *C. lunata* (Wakk) Boed., its characters being in full agreement with the description of the species given on different hosts by Subramanian (1953), Rao and Salam (1954) and Luttrell (1956).

The material of sooty black Bajra grains received from Rao and Salam (Hyderabad) was examined and the size and shape of the conidia of the two specimens compared. No significant differences were observed between the two fungi (Fig. 1 and Table 6).

Agarwal (1958) made nutritional studies of the fungus named as *C. penniseti* obtained from Bajra seeds. A culture of this fungus was obtained from Allahabad University culture collection and its characters compared with those of the culture under study. The size, shape and thickness of the wall of conidia in the two cultures were found to be more or less identical (Fig. 1 and Table 6). Moreover, their similar behaviour in physiological characters as studied by the authors and reported by Agarwal (1958) *i.e.* optimum temperature for the growth of the culture (28°C.); capacity of utilization of different nitrogenous compounds especially in-

ability to grow on ammonical inorganic salts; and growth on different carbon compounds, confirmed the view that the two were indentical.

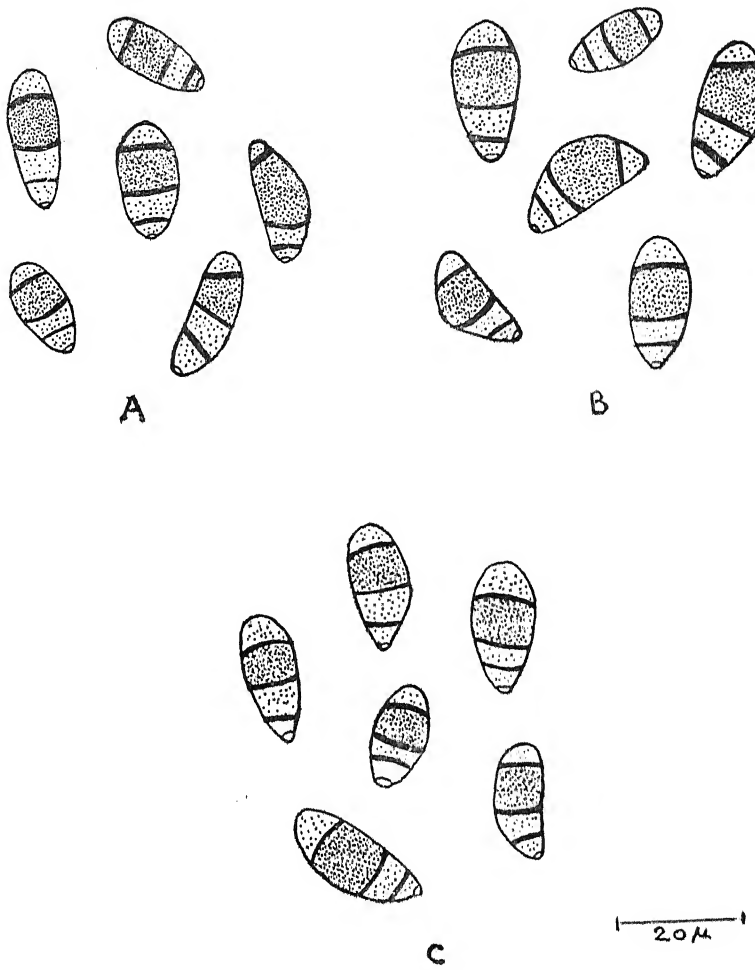


Fig. 1

Conidia of the three cultures of *Curvularia* from *Pennisetum typhoides*.

(A) *C. Penniseti* (Allahabad)

(B) *C. Penniseti* (Hyderabad)

(C) *C. lunata* (Under study)

TABLE 6

Comparative size of Conidia from the three cultures of *Curvularia* from Bajra

	Source of Material	Size of conidia in μ			
		Length		Breadth	
		Range	Average	Range	Average
(*a*) *C. penniseti*	Allahabad University culture collection	18·4 – 23·4	21·9	8·3 – 13·4	10·5
(*b*) *C. penniseti*	Hyderabad	16·2 – 27·0	21·5	8·0 – 14·4	11·5
(*c*) *C. lunata*	Material under study	18·0 – 28·8	23·4	8·0 – 14·4	10·9

The slightly larger size of conidia of *C. penniseti* reported by Mitra (1921) and Rao and Salam (1954) can conveniently be covered within the range of 15·2 to 42 μ × 7·6 to 14·3 μ, the dimension of conidia of *C. lunata* described by Luttrell (1958) from different hosts.

The authors are, therefore, of the view that the fungus causing the sooty mould of Bajra grains be identified as *C. lunata* (Wakk) Boed., and *C. penniseti* (Mitra) Boed., be dropped as a distinct species and considered a synonym of *C. lunata.*

SUMMARY

(1) Blackening of individual grain in ear heads of Bajra was commonly observed in August and September in Rajasthan.

(2) Causal organism was identified as *Curvularia lunata* (Wakk) Boed., and the pathogenicity of the fungus was established.

(3) Variation in spore size was observed on the different media used for morphological studies.

(4) Physiological studies of the fungus should that :—

(*a*) Optimum temparature and pH was 28°C and 6·5 respectively.

(*b*) Among nitrogenous compounds studied, asparagin gave the best growth and sporulation, while ammonical inorganic salts were not utilised.

(*c*) Out of the carbon compounds used, glucose and sorbitol produced the best growth and the former best sporulation also. Fructose supported poorest growth and sporulation.

(5) On the basis of morphological and physiological characters, *C. penniseti* has been found to be a synonym of *C. lunata* causing blackening of Bajra grains.

ACKNOWLEDGMENT

Authors are grateful to Dr. N. Prasad, Plant Pathologist, Rajasthan for guidance, to Dr. M. B. Ellis of Commonwealth Mycological Institute for indentification of *C. lunata* and to Shri Samarth Raj, Director of Agriculture, Rajasthan for facilities. Thanks are also due to Dr. M. A. Salam and Dr. R. N. Tandon for making the diseased specimen and culture of *C. penniseti* available for study.

REFERENCES

1. Agarwal G. P. (1958), Nutritional studies on *Curvularia penniseti*, II, carbon and nitrogen requirements. *Phyton* 11 (2): 143—151, XII.

2. Luttrell, E. S. (1956), The separation of *Curvularia trifoli* and *C. lunata*. *Plant Dis. Reptr.* 40, 1, 57—60.

*3. Mitra, M. (1921), Morphology and Parasitism of *Acrothecium penniseti* n. spp. (A new disease of *Pennisetum typhoideum*). *Memoirs Dept. Agric. Ind. Bot. Ser.* XI, 3, 57—74.

4. Rao, P. N. & Salam, M. A. (1954), *Curvularia* species from discoloured grains from Hydarabad-Dn., *J. Indian Bot. Soc.* 34 : 268—71.

5. Subramanian, C. V. (1953), Fungi Imperfecti from Madras V. (*Curvularia*). *Proc. Indian Acad. Sci.*, B, 38: 27—39.

*Original not seen.

A NEW SPECIES OF PHOMA ON THE PHYLLOCLADES OF *MUEHLENBECKIA PLATYCLADOS*

By

R. N. TANDON *and* K. S. BILGRAMI

Botany Department, Allahabad University

[Received on 15th June, 1960.]

The phylloclades of *Muehlenbeckia platyclados* were found to be severely infected at Allahabad. Light brown spots which later changed to dark grey colour were produced over the host. Generally the infection started from the tip but some scattered lesions were also developed. In severely diseased regions black pycnidia were distinctly visible (vide fig. 1). Sometimes the healthy and infected regions

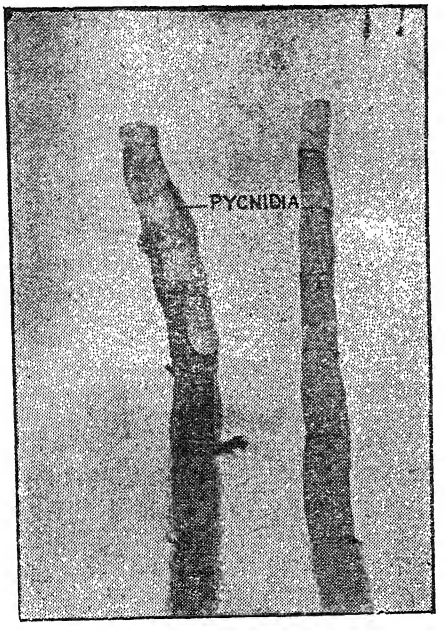


Fig. 1—Showing black dot like pycnidia developed in the infected region.

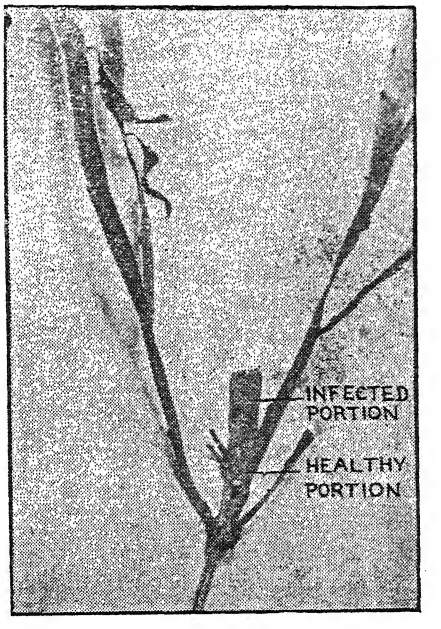


Fig. 2—Showing healthy and infected portions of the phylloclade separated by a light brown band.

were separated by a light brown band (vide fig. 2). The tissues from the tip become dry and brittle at advanced stage of infection and ultimately they may fall off. The disease occured practically throughout the year but severest infection was noticed from 3rd week of October to February. Cold weather accompanied by slight moisture favoured the development of the disease.

Morphological studies—Young hyphae are hyaline and sparsely septate, while the older ones are light brown in colour with very frequent septa (3·5—4·7 μ in breadth). Some of the hyphae are abnormally thick and attain a width of about 7 μ. Chlamydo-spores of various shapes and size are frequently developed in old cultures of 20 days or more in age. Pycnidia are generally separate, globose

ostiolate, dark brown or black in colour. Their size ranges from 140 – 152 × 116 – 131 μ. Major portion of their body is embedded inside the host tissue, only a small portion pierces through the epidermis ; conidiphores are very short ; spores are single celled subhyaline and ovate. Their size ranges from 10 – 13 ×4·5 – 5 μ (vide fig. 3).

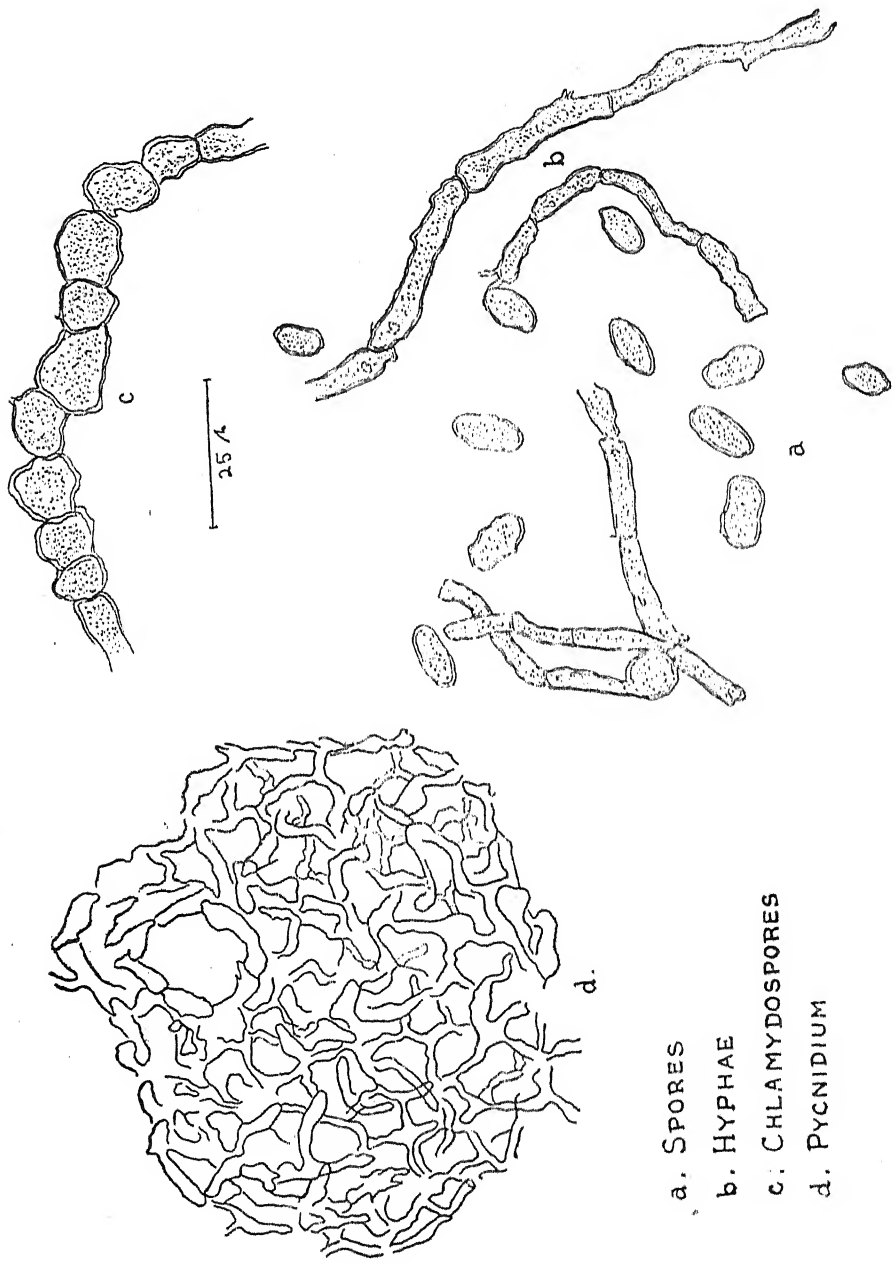


Fig. 3—Showing spores, chlamydospores, hyphae and pycnidium.

A large number of single spore isolations were made from the host, they were identical in every case. On the basis of morphological and cultural studies it was concluded that the fungus was some species of *Phoma*. A species of this fungus, *viz.*, *P. muehlenbeckiae* (Cooke *et* Mass) has been reported on *Muehlenbeckia* sp. from kew[1]. A comparison of the present species of *Phoma* with *P. muehlenbeckiae* showed marked differences which are detailed in table 1.

TABLE 1

Representing the differences in the spores and pycnidia of *P. muehlenbeckiae* and the present species of *Phoma*.

Organism	Spores				Pycnidia
	Length	Breadth	Shape	Colour	
P. muehlenbeckiae	2·5 μ	0·5 - 0·7 μ	rod like	hyaline	usually in clusters
Present species	10 – 13 μ	4·5 - 5 μ	ovate	subhyaline	usually separate

Besides the above mentioned differences, *P. muehlenbeckiae* was recorded on the dead tissues of *Muehlenbeckia* sp. while the present species of *Phoma* was isolated from the living phylloclades of the host and the pathogenicity of the organism was established by spraying a spore suspension.

The description of all those species of *Phoma* which have bigger spores was compared but it did not agree with any of those. It is, therefore, concluded that the organism is some new species of *Phoma*, which is pathogenic in nature. It is proposed to name it as *P. allahabadense* —a new species. Latin description is given below :—

"Pycnidia fusce brunnea, ostiolata *et* globosa, 140 - 152 × 116 - 131 μ, immersa in plantam hospitem, rostro brevi emergente per epidermidem. Condiophori brevissimi; sporae ex una cellula constantes, subhyalinae *et* ovatae, 10 – 13 × 4·5 - 5 μ.

Species haec a *Ph. muehlenbeckiae* eminenter differt magnitudine, forma *et* colors sporarum atque dispositione pycnidiorum, ad nullam vero aliam speciem cognitam generia *Phoma*; accedit, quapropter hic nominatur *P. allahabadense* spec. nov."

The authors are grateful to Prof. H. Santapau of St. Xavier's College, Bombay for translating the description in latin.

REFERENCE

1. Saccardo, P. A.—*Sylloge fungorum* 1892, **10** : 157

HISTOLOGICAL STUDY OF THE KIDNEY OF OPHICEPHALUS MARULIUS AND OPHICEPHALUS STRIATUS

By

S. K. DUTTA *and* S. RAJAM

Zoology Department, University of Allahabad, Allahabad

[Received on 15th October, 1958]

INTRODUCTION

Recently the studies of the kidneys of the fishes have drawn a good deal of attention of different zoologists and biochemists. Because, other than the anatomical and histological studies of the organ which indicates a good deal about the nature of the evolution the organism has followed, it also throws light on the evolution of, nitrogen metabolism, in the shape of elaboration of amino-acids, of the kidney. The kidney plays prominent role in the water-balance, and nitrogen metabolism of the organism through the investigation of the kidney of fishes—an organism having vertebrate kidney and yet living exclusively in water—we get a glimpse of the evolution of the nitrogen metabolism system and the utility of the water-balance system in the animal kingdom.

Here in this paper we have studied the kidneys of *Ophicephalus marulius* and *Ophicephalus striatus* and have observed that the kidneys of these fishes have specific localised cell-patches which contain chemicals, some of which take dark colour with haematoxylin-eosin stain and some retain the original dark brown colour. Edwards (1) has observed similar dark coloured patches in marine and fresh-water fishes. These coloured patches are significant and so far no one has thrown any light on it. Edwards has only hinted that it is probably a type of mucus secreting cells which does not take up any stain at all. These brown spots have materials which act as inhibitor or activator of the various proteolytic reactions; which take place in the kidney. We have observed that in certain fishes, when blood goes to the kidney it is poor in amino acids and contain only a few amino acids, but when it comes out of kidney it is fairly rich in amino acids and several new amino acids are synthesised. This work is under print elsewhere.

It appears that the glands observed in the kidney of the fishes studied here also play a great role in the nitrogen eonomy of fishes.

Description of the kidney of Ophicephalus marulius :

The pronephros of *Ophicephalus morulius* as examined in transverse sections, indicates a large number of hexagonal cells containing a colourless fluid as observed after treating the material with fixative and which stains red with haematoxylin and eosin (Fig. no. 1). The red staining cells are present in groups and each group is covered with a double-walled coating. The cells of the outer layer are elongated, resembling muscle strands and, containing one small nucleus in each cell. The inner layer is of two-cell thickness and below this is present another layer of two-cell thickness. The cells of this outer layer stain dark red with haematoxyline and eosin and show big prominent dark-blue nuclei. These two-layered coats encircle the big cells which stain pink with haematoxalyne and eosin. Such bundles of large cells

stainable red with haematoxylin are found plentifully in each section of the pronephros.

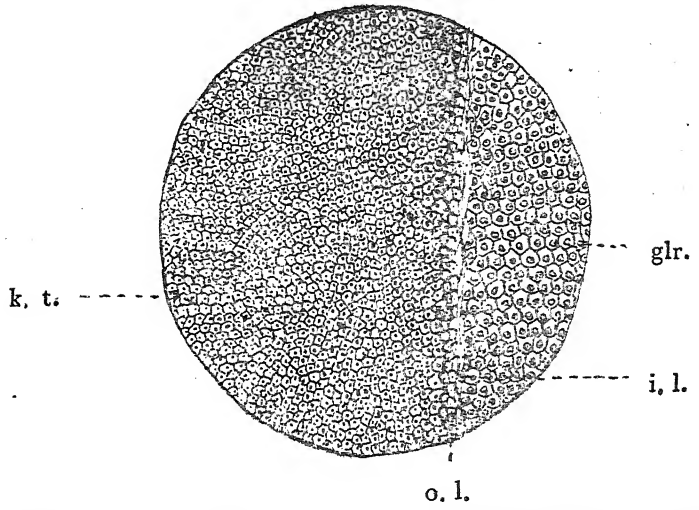


Fig. 1. Diagram of a section through the pronephros region of Ophicephalus marulius (10 × High Power).

[K.t.—The kidney tissue ; gl.r.—The cells of the gland-like red region ; i.l.—The inner layer]

Mesonephros:—The mesonephros region of the *Ophicephalus marulius* shows a very small number of glomeruli (Fig. no. 2). The number is so meagre that hardly one or two glomeruli may be seen in each field of vision. The glomeruli are large in diameter measuring 15μ with capillaries arranged in elongated pattern.

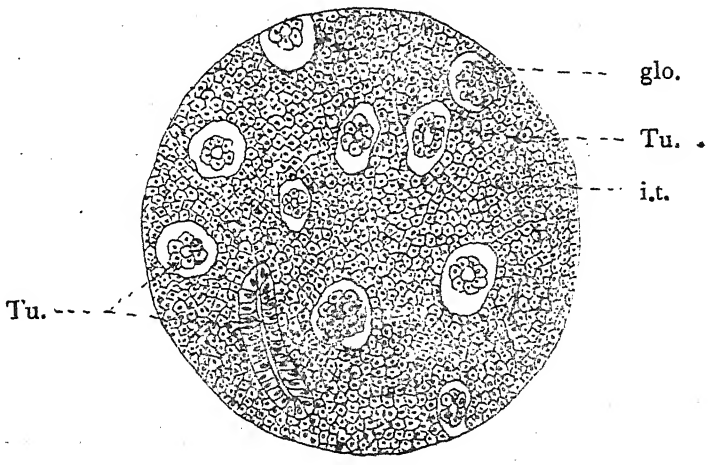


Fig. 2. A section through the anterior portion of mesonephros of Ophicephalus marulius (10 × High Power).

[Tu.—Tubules ; glo.—glomerulus ; i.t.—Inter-tubular tissue]

The mesonephros region of *Ophicaphalus marulius* contains a large number of tubules as compared to other fishes. The cells lining these tubules are long with prominent nuclei.

The mesonephros contains some inter-tubular cells staining pink with haematoxylin and eosin. The nuclei of these inter-tubular cells stain blue with the above stain and are big in size. There are other types of inter-tubular cells which contain histologically different cells staining red with haematoxyline and eosin. These are unlike the typical inter-tubular cells and possess small nuclei.

Lower portion of the mesonephros :—The lower portion of mesonephros contains still fewer glomeruli, but these are much bigger in size, 20 to 21μ as compared to the glomeruli of the upper and middle portions of the mesonophros (Fig. no. 3).

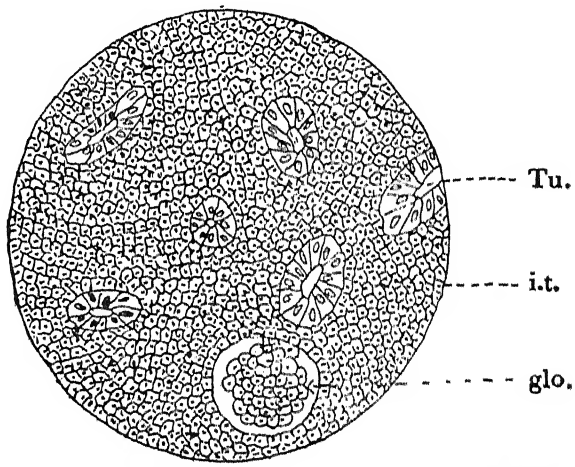


Fig. 3. A section through the posterior region of mesonephros of Ophicephalus marulius (10 × High Power).

[Tu.—Tubule ; i.t.—Inter-tubular cells ; glo.—glomerulus]

The capillaries of the glomeruli are bigger than those in the glomeruli of this upper region, but here these capillaries are longer and arranged in an oval pattern. This region of the kidney contains a large number of tubules which are similar to the upper and middle portion of the mesonephros with the only difference that the number of tubules are more than those in the upper region.

The inter-tubular cells are few in number but are of the same type as observed in this upper and middle portion of mesonephros. The number of red patches developed after haematoxylin and eosin stain is also fewer than those in the upper portion.

This in short we see that the kidney of *Ophicephalus marulius* is peculiar in the following respects :—

(1) The pronephros region is devoid of glomeruli and tubules but possesses red stained hexagonal cells.

(2) Amongst the intertubular cells are present a type of cells which have smaller nuclei and which stain red with haematoxyline and eosin.

The cells are arranged in bundles, which have double layered coating. These cells are in larger number in pronephros and the number decreases as we go towards the posterior portion of the kidney where the number of glomeruli is much less, but are comparatively bigger in size.

(3) The upper portion of mesonephors contains a larger number of tubules than those found in the kidney of other fishes. The large number of tubules in this region is due to the fact that some of the tubules of this posterior portion, where they had been before, are forced to this region may be due to shortage of space in the lower portion.

It has been observed that the kidney of *Ophicephalus marulius* behaves as an organ for the elaboration of amino-acid synthesis. The blood, as it enters the kidney, contains glutamic and aspartic acid in a large quantity, but when it leaves the kidney it has been found to be rich in histidine, ornithine, arginine and other amino acids. It appears that the intertubular cell, which stains pink with heamatoxylin and eosin stain appears to behave like glands of internal secretion and contains the engymic systems which help in the synthesis of the amino acids.

There seems to be much economy in the nitrogen excretion of *Ophicephalus marulius*, for the lessor number of glomeruli with fewer capillaries in the anterior portion of mesonephros indicates that only a small quantity of the waste nitrogenous matter is excreted out in the form of urea and uric acid. Most of the nitrogenous substance is used up in the synthesis of amino acids. Thls fish is not only capable of living outside the water with the help of its accessory respiratory organ, but also has an unique economy in its nitrogen metabolism, for it can neutralize the waste nitrogen by synthesising amino acids, which are of vital importance for its normal life activity.

DESCRIPTION OF OPHICEPHALUS STRAITUS

Pronephros:—The pronephros of *Ophicephalus striatus* has no glomerul (Fig. no. 4). A section of the pronephros shows patches of cells which stain pink

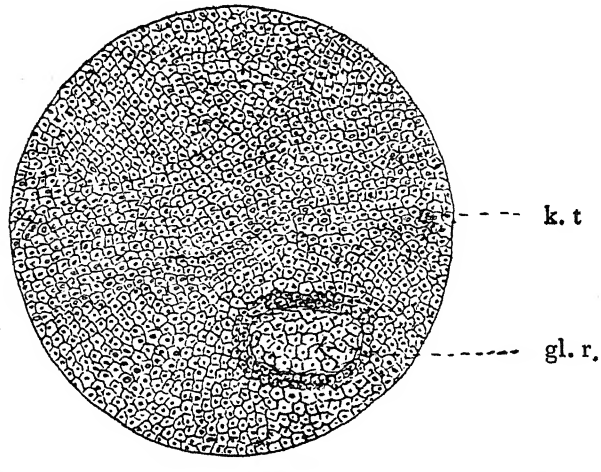


Fig. 4. Diagram of a section passing through the pronephros region of Ophicephalus striatus (10×High Power).

[K.t.—Kidney tissue; gl.r.—gland like red region with the coats]

with haematoxylin and eosin stain. The size of these pink patches is smaller than the size of those observed in the pronephros zone of *Ophicephalus morulius*. The pink cells of *Ophiocephalus striatus* have a coating of double walled cells which are similar to the coating of the pink cells observed in *Ophicephalus morulius*. The shape of these cells staining pink with haematoxylin eosin stain is irregular. The double layers of this tissue are each of two cells thickness. The cells of the outer layer stain dark red with haematoxylin eosin stain and their nuclei appear dark blue in colour and are very prominent.

Anterior mesonephros :—The size of glomeruli in the anterior portion of mesonephros vary from 12 μ to 20 μ. Only a few tubules are observed in this zone, the greater part being occupied by the intertubular cells (Fig. no. 5). The anterior

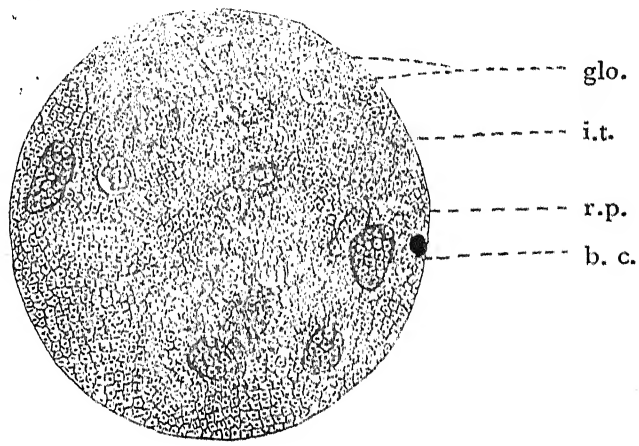


Fig. 5. The section passing through the anterior mesonephros of Ophicephalus striatus.

[glo.—glomerulus ; i.t.—Inter-tubular cells ; r.p.—red patch ; b.c.—brown cells]

region of the mesonephros shows several patches of specific cells staining dark-red with haematoxyline and eosin as observed in the case of pronephric region. The cellular aggregates no longer remain oval but acquire different shapes.

Together with the intertubular cells staining dark pink with haematoxylin and eosin stains, there is another type of cellular glands whose cells remain brown, the original colour, even after staining with above stain. Only a small number of inter-cellular tissues is observed in this region; also a few tubules are seen. The packing cells are big in size and have prominent nuclei.

Posterior mesonephros :—The posterior region of the mesonephros shows a few very big glomeruli measuring 20 μ, which are bullus in shape. The capillaries are big with small nuclei (Fig. no. 6). The Bowman's capsule has a definite outline.

The number of tubules is very small in the posterior region of the mesonephros and their cells appear square in shape, with small nuclei. The canal of the tubules are broad.

The intertubular glands comprising the cells staining dark pink and dark brown with haematoxylin eosin stain, are found in abundance in the posierior region of the mesonephros. But the size of the red-celled glands decreases

whereas the size of the brown-celled glands increases in this region. The brown celled glands are about $2\frac{1}{3}$ times μ of the red cells.

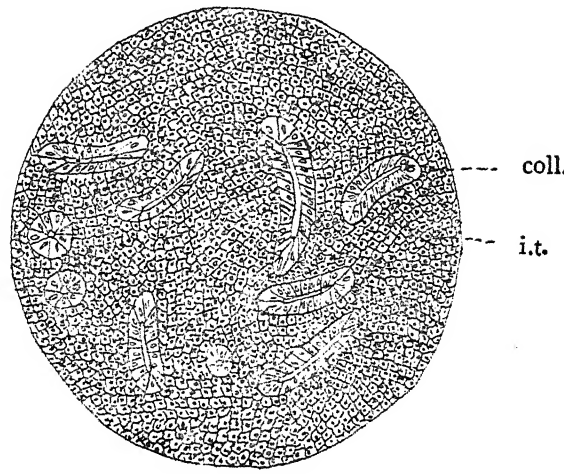


Fig. 6. A section passing through the posterior mesonephros of Ophicephalus striatus.
[Coll.—Collecting tubule; i.t.—Inter-tubular cells]

Thus the kidney of *Ophicephalus stratus* is characteristic in the following respects:—

(1) The number of glemeruli is very few and their size is very small.

(2) The mesonephros contains two types of glands. The cells of one stain dark red with haematoxylin eosin stain and those of the other does not take up any stain at all and retain with their original dark brown colour.

(3) The number of tubule is small and intertubular cells are larger in dimension.

DISCUSSION

The fishes of Ophiocephalideae family show a high degree of adaptation. Being a fresh water fish, they have to undergo a lot of hardship during the drying of the ponds and most of these fishes lived in scanty water during the summer. The fishes of this family have developed an accessory breathing apparatus which enables them to live for a fairly longer period outside water or in an environment where water is scarce. They have not only developed the accessory breathing apparatus but also developed a high degree of economy in its nitrogen metabolism. The results about the synthesis of amino acids in the kidney of these fishes will be published elsewhere.

The intertubular glands of the kidney help to synthesize amino acids.

Ophiocephalus striatus shows greater adaptations to the environment than *Ophicephalis morulius* as is indicated by the presence of dark greenish black stripes on its body which provides it greater chance of hiding from its enemy by its resemblance to the surroundings. The better adaptation is not only external but is also seen in the inner structure particularly in the kidney which indicates very few apparatus for the extraction of urine and urea from the blood.

CARBON REQUIREMENTS OF SOME MEMBERS OF THE FAMILY SAPROLEGNIACEAE*

By

RAM DAYAL

Department of Botany, C. M. P. Degree College, Allahabad

[Received on 4th July, 1960]

INTRODUCTION

Amongst the essential elements required by living organisms carbon occupies the premier position. It has now been proved that the greater bulk of the fungus mycelium consists of carbon which is present in it in various forms. For the growth of fungi in general, it has been seen that carbohydrates usually prove to be the best sources. The response of fungi towards various carbon sources may be so specific that one substance may be utilized while another of closely similar chemical structure may prove to be useless. The example of such a specificity has been shown by Lilly and Barnett (1956) for the utilization of D-arabinose by *Sporobolomyces salmonicolor*. They reported that this fungus grew well on D-arabinose and failed to grow on L-arabinose.

Some studies have been made in the past regarding the suitability of different carbon compounds for the members of the family Saprolegniaceae. Pieters (1915) reported that *Achlya racemosa, A. prolifera, Saprolegnia ferax* and *S. monoica* were unable to utilize sucrose. Saksena (1940) found dextrose, maltose, sucrose and starch to be good carbon sources for some species of the genus *Pythium*. Bhargava (1945) studied the growth of *Achlya* sp., *Brevilegnia gracilis, Isoachlya* sp, *Saprolegnia delica*, and *S. monoica* on different carbohydrates and sugars. He reported that glucose, fructose, maltose and starch were the best carbon sources, while mannose served as an equally good source for *B. gracilis* and *S. monoica*. Similarly Bilgrami (1956) and Raizada (1957) have reported some carbon compounds which were suitably utilized by the organism studied by them.

The investigations reported herein were carried on to determine by quantitative measurements the relative growth supporting values of several different carbon compounds for the following members of the family saprolegniaceae viz., *Achlya aplanes*, Maurizio var. *indica, Isoachlya unispora* Coker and Couch, *Isoachlya toruloides* Kauffman and Coker and *Saprolegnia parasitica* Coker.

MATERIAL AND METHODS

Unless otherwise stated, various carbon compounds were added to the basal medium† so as to give 2000 mgm. of carbon per litre. Starch was taken equal in weight to glucose due to its unknown constitution. All solutions were autoclaved at 15 lbs. pressure for 15 minutes except those containing oligo-and polysaccharides. Such solutions were steamed for 15 minutes at no pressure for three consecutive

* Part of the thesis approved for the degree of Doctor of Philosophy in the University of Allahabad.

†KH_2PO_4 0·5 gm. $MgCl_2 \cdot 6H_20$ 0·5 gm. NH_4NO_3 2·0 gm. Na_2S 0·17 gm. and distilled water 1000 ml.

days in order to avoid the hydrolysis of these compounds at high pressure. The pH of the media was adjusted to 7·0 before autoclaving. Material for the inoculation was taken from the actively growing colony and the inoculations were made by agar disc method.

EXPERIMENTAL

Various media were inoculated with *Achlya aplanes*, *Isoachlya unispora*, *I. toruloides* and *Saprolegnia parasitica*. Basal medium alone served as control. The dry weights given in Table 1 are presented after statistical analyses of the data.

Table 1 : Showing the dry weight (in mgm.) of four fungi on media containing equivalent quantities of different carbon compounds.

Fungi	Carbohydrates.				
	Glucose	Fructose	Maltose	Starch	Glycerol
Achlya aplanes	16·7	15·4	21·3	18·4	9·0
Isoachlya unispora	20·6	18·4	25·0	21·0	4·0
Isoachlya toruloides	18·7	17·0	20·3	24·0	7·0
Saprolegnia parasitica	19·6	17·3	24·7	23·0	12·0

A careful study of the résumé presented in the above mentioned table indicates that amongst the monosaccharides pentoses did not support growth of the organisms under study.

The behaviour of hexoses was not similar towards all the fungi. Glucose and fructose proved to be good sources of carbon for all the organisms, while galactose proved to be valueless.

Of the four disaccharides maltose supported good growth of all the fungi. Sucrose, lactose and melibiose proved valueless as sources of carbon for the organisms under investigation.

Raffinose was useless for the growth of all the fungi.

Starch was an excellent source of carbon for the growth of all the organisms.

The behaviour of two sugar alcohols, viz., glycerol and dulcitol was different towards various fungi. Glycerol was a good carbon source for all the organisms except *I. unispora* for which it proved poor. Dulcitol could not support any growth of the fungi.

Tartaric acid was found useless source of carbon for the growth of the organisms.

*Arabinose, rhamnose, xylose, galactose, lactose, sucrose, melibiose, raffinose, dulcitol, and control were not utilized.

DISCUSSION

The results clearly show that all fungi are not capable of utilizing same sugars with the same efficiency. The simple carbon compounds are assimilated directly while the complex ones are converted into simpler forms before their utilization. For the first part the configuration of the carbon compounds plays an important role, while for the other production of the necessary hydrolytic enzymes is essential. The utilization of monosaccharides is usually assumed to be direct and independent of the carbohydrases required to hydrolyze the complex carbohydrates prior to their actual utilization.

Pentoses (arabinose, rhamnose and xylose) have been generally reported to be poor sources of carbon by several authors. In the present investigations also pentoses did not support growth of the organisms. Of the hexoses glucose and fructose were found to be equally good sources for all the organisms while galactose, on the other hand, was valueless. The good growth of the fungi on glucose and fructose is probably due to their chemical similarity (having same configuration for carbons 3-6), having same enolic form and producing the same hexose phosphate. Galactose differs from these two sugars (glucose and fructose) in the above respects. The author agrees with the view of Blank and Talley (1941) who have suggested that the chemical configuration and properties of galactose prevent it from being a readily available carbon source.

Of the four disaccharides used, maltose served as a good carbon source for all the fungi investigated by the author, while sucrose, lactose and melibiose were valueless. Here a specificity has been shown by the organisms for the utilization of the different disaccharides. This has depended on the ability of the organisms to hydrolyze these disaccharides into monosaccharides. Maltose consists of glucose units and it has been shown by the author in a separate experiment that this sugar was easily broken down by the organisms and that the resulting hexose sugar may responsible for their good growth. The absence of growth on sucrose, lactose and melibiose may be explained on the basis of the nonsecretion of necessary hydrolytic enzymes by the fungi. The statement finds further support by the chromatographic studies made by the author on the utilization of oligo-and polysaccharides.

Raffinose is probably not being utilized due to the inability of the various fungi to secrete necessary enzyme. This assumption has been supported by the fact that all the fungi are incapable of hydrolyzing this sugar as reported by the author (Ram Dayal, 1958). Bhargava (1943) also observed that raffinase was not secreted by some organisms of the family Saprolegniaceae.

Starch was well utilized by the fungi. According to Blank and Talley (1941), "the ease of utilization of polysaccharide seems to be directly correlated with the rate at which these particular polysaccharides are converted into simpler carbohydrates by the organisms." That starch was hydrolyzed into maltose and glucose by the fungi under investigation has been shown by the author in a separate experiment. The good utilization of starch agrees very well with the findings of Volkonsky (1934) who found that it was well utilized by 26 isolates and species of the Saprolegniales.

Glycerol was found to be a good source of carbon for *Achlya aplanes* and *Saprolegnia parasitica* but mediocre for the two species of *Isoachlya*. Similar results have been reported by Volkonsky (1933) for *Pythium debaryanum*, by Saksena (1940) for some species of *Pythium* and by Mehrotra (1949) for some species of *Phytophthoras*. Dulcitol proved to be useless for the growth of the organisms. Similar results were reported by Bhargava (1945) for some members of the family Saprolegniaceae.

Lilly and Barnett (1951) are of the opinion that generally the organic acids do not allow the fungi to grow better than on carbohydrates. Tartaric acid was not utilized by any of the fungi tested by the author. These results are also in agreement with the findings of Bilgrami (1956) and Raizada (1957) for the fungi investigated by them.

SUMMARY

1. The general suitability of different carbon sources viz., sugars, sugar alcohols and organic acids, for the growth of *Achlya aplanes*, *Isoachlya unispora*, *I. toruloides* and *Saprolegnia parasitica* was studied.

2. Of the monosaccharides pentoses proved to be usel ss for all the fungi. Hexoses (except galactose) were most favourably utilized.

3. Amongst the disaccharides maltose proved to be a good carbon source, while sucrose, lactose and melibiose were valueless for the fungi.

4. Raffinose proved useless for the organisms.

5. Soluble starch was found to be a good source of carbon.

6. Glycerol was utilized in varying degrees, while dulcitol proved valueless.

7. Tartaric acid proved to be a useless source.

ACKNOWLEDGEMENTS

The author is grateful to Dr. R. K. Saksena, Ex-Professor of Botany, University of Allahabad for his keen interest and guidance in this work. Thanks are also due to Dr. K. S. Bilgrami and Dr. B. B. S. Raizada for their help in many ways.

LITERATURE CITED

1. Bhargava, K. S., 1943, Physiological studies of some members of the family Saprolegniaceae. I. Enzyme action. *Jour. Ind. Bot. Soc.*, **22**, 85-99.

2. ———————— 1945, D. Phil. Thesis University of Allahabad.

3. Blank, L. H. and P. J. Talley, 1941, The carbon utilization and carbohydrate activity of *Phymatotrichum omnivorum*, *Amer. Jour. Bot.*, **28** ; 564-569.

4. Bilgrami, K. S., 1956, Pathological and Physiological studies of some fungi causing leaf spot diseases. D. Phil. Thesis, University of Allahabad.

5. Lilly, V. G. and H. L. Barnett, 1951, Physiology of the fungi. McGraw Hill Publications in Bot. Series.

6. ———————— 1956, The utilization of D-and L-arabinose by fungi. *Amer. Jour, Bot.* **43**, 709-713.

7. Mehrotra, B. S., 1949, Studies on some *Phytophthoras* and soil fungi. D. Phil. Thesis, University of Allahabad.

8. Pieters, A. J., 1915, The relation between vegetative vigour and reproduction in some Saprolegniaceae. *Amer. Jour. Bot.* **2**, 529-576.

9. Raizada, B. B. S., 1957, Physiological, cytological and taxonomic-studies of some Mucorales. D. Phil. Thesis, University of Allahabad.

10. Ram Dayal, 1958, Studies on some aquatic fungi. D. Phil. Thesis. University of Allahabad.

11. Saksena, R. K., 1940, The nutrition of some species of the genus *Pythium* on synthetic liquid media. *Proc. Nat. Acad. Sci. India*, **10**; 1-13.

12.* Volkonsky, M., 1933, Sur les conditions de culture et le pauvoir de synthése de *Saprolegnia* sp. E'tude qualitative de l'alimentation. *Ann. Inst. Pasteur* **50**; 703-730.

13.* ———————— 1934, Sur la nutrition de quelques champignons saprophytes et parasites, *Ann. Inst. Pasteur*, Paris, **52**; 76-191.

* Papers not seen in original.

STUDY OF THE PRONEPHRIC KIDNEY OF WALLAGONIA ATTU

By

S. RAJAM *and* KRISHNA BAHADUR

Departments of Zeology and Chemistry, Allahabad University, Allahabad

[Received on 15th October, 1958]

Marshall and Smith (1) have reported that a larger number of glomeruli in the Kidney is indicative of fresh water habit of fishes and decrease in the number and size of glomeruli, increase of connective tissues and increase in tubule formation indicate that the fish has developed a mechanism of water economy due to its sea-water habitation. The decrease of glomeruli and increase of connective tissues indicate that the organism has developed a system of excreting out the nitrogen and mineral metabolites without much loss of water.

In most of the fishes studied, the mesonephros is usually found to be the functional kidney and it contains varying number of glomeruli depending upon the species of the fish, connective tissues and tubules and this is the chief organ of excretion. In the posterior portion of the fish kidney the number of tubules increases and the number of glomeruli is considerably decreased. In most of the fishes studied so far with the exception of a few, pronephros is non-functional and devoid of any glomerulus.

Pronephros is functional in cyclostomes. The presence of functional pronephros in certain fishes as *Wallagonia attu*, whose description we will consider in this paper, indicates the lineage of the class of fishes from primitive vertebrates. We have observed that the pronephros of *Wallagonia attu* contains a single pair of glomeruli. The number of glomeruli remains large in mesonephros and a few of them can also be seen in the posterior part of the mesonephros. A detailed study of the kidney of *Wallagonia attu* is given below.

Pronephore :—A section of the pronephros indicates the presence of a large number of prominently nucleated polygonal connective cells and among these cells are persent a pair of big prominent well vascularised glomeruli of about 41 μ in cross section. The glomeruli consist of a fine net work of capillaries surrounded by a space which contains urinary fluid. However, other than this solitary pair of prominent glomeruli, there is no glomerulus or tubule in the pronephros region (Fig. no. 1).

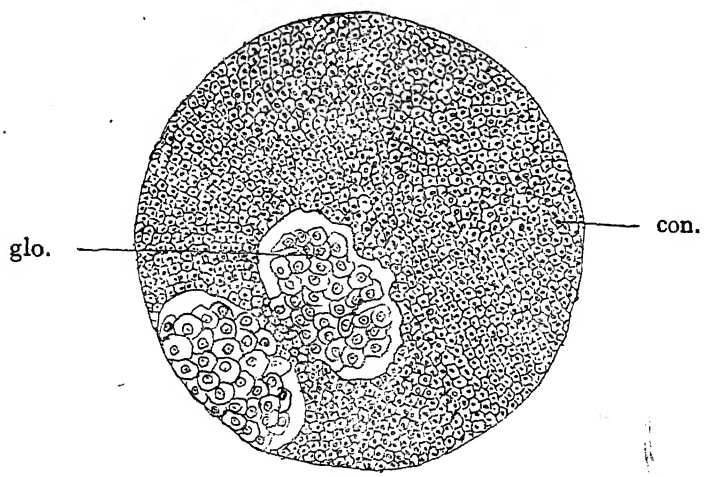


Fig. 1. Section passing through the anterior end of the Kidney of *Wallagonia attu* (12 × High Power).

[Con.—Connective tissue; glo.—The only pair of glomeruli]

The well developed structure of pronephros is indicative of the functional pronephros in this fish.

Mesonephros:—Mesonephros is also thoroughly filled with connective tissues in which are embeded a few glomeruli ranging from 16 μ to 20 μ. The number of glomeruli per sq. m. m. as counted from the sections have been found to be about six (Fig. no. 2). This region is fairly rich in tubules of medium size but of verying shapes. The cells of the tubules are regular with prominent nuelei.

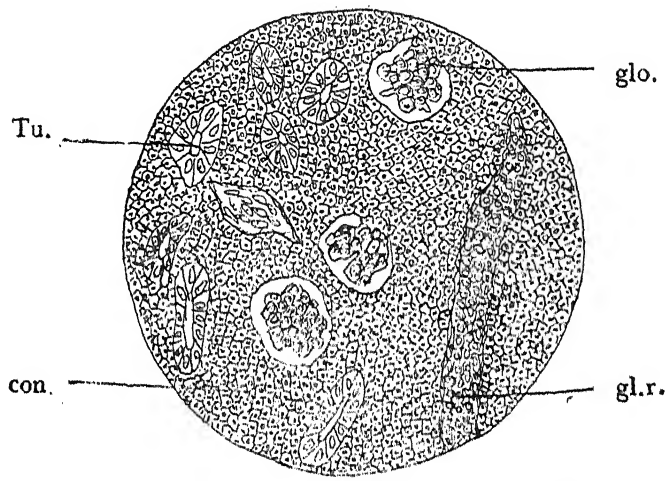


Fig. 2. Section passing through the anterior mesonephros of *Wallagonia attu* (12 × High Power).
[Tu.—Tubule; glo.—glomerulus; con.—Connective tissue; gl.r.—glandular red cells]

Posterior region of mesonephros:—The cross section of this region shows smaller number of glomeruli than that of the anterior mesonephros (Fig. no. 3). They

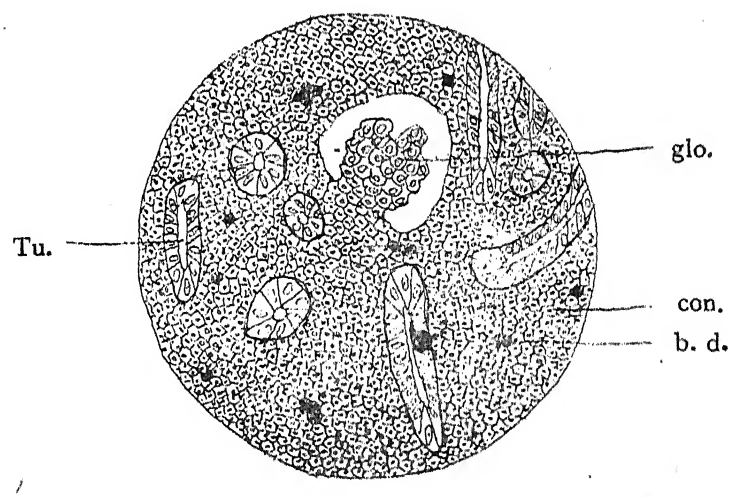


Fig. 3. Section passing through the posterior mesonephros of *Wallagonia attu* (12 × High Power).
[Tu.—Tubule, glo.—glomerulus; con.—connective tissue; b.d.—brown deposit]

range in size from 23 to 29 μ. This seems to be in confirmation with the view of Audige (2) that if glomeruli are found to be present in the posterior part of the kidney, they are usually bigger in size. This region contains a large number of tubules with rectangular nucleated cells. The connective tissues are polygonal in shape and well nucleated.

DISCUSSION

Though in most of the fishes mesonephros is functional and the pronephros which is functional in lower vertebrates, remains nonfunctional yet in a few fishes as *Aplocheilus melastigma* described by Rangarajan (3) the pronephros has been found to be functional. The presence of a pair of prominent glomeruli in this pronephros region and presence of fairly big glomeruli in the anterior and posterior mesonephros region is in agreement with the fresh water habit of *Wallagonia attu.* Marshall (4) has reported that the fishes which have fresh water environment have large number of glomerule for the excretion of the excess of water which they take in together with their food and which mostly enters their body by the process of osmosis through the skin.

It appears that the functional pronephros is indicative of the fact that this animal has been recently derived from his ancestors which had a functional pronephros. But as in most of the fishes and in the higher vertebrates as amphibians and reptiles we find the pronephros is either the larval kidney or completely absent, it appears that there was an independent line of evolution from the lower-vertebrates with prominent functional pronephros to fishes of Siluroideae family. This is further supported by the fact that unlike the fishes of Cryprinoideae, Dupioideae and others, this family is completely devoid of scales, have a prominent mouth, and Nature tried to protect these animals by providing them with long barbules and the fishes of this family in the process of struggle through environments developed herbivorous habits as is indicated by the fact that all the fishes of Siluroidae family have premaxillae. The great effort to fight the unfavourable conditions by this family is also indicated by the fact that some of the fishes of this family have got prominent trilobed air-bladders (5) and a few of these fishes have even developed an accessory respiratory organ as in *Hetropneustes* and in *Clarias.* But inspite of all these adaptations to the environment, this fish is not suited for the marine habitat, because of the large number of glomeruli in the kidney.

The absence of pronephros in amphibians' indicates that they did not evolve from any of the fishes of Silurideae and so we can very well say that the line of evolution started from the ancestors with functional pronephros and it led to Silurideae family when fishes were without scales and thus comparatively unprotected from their enemies and they developed extra sensory organs as barbules to protect themselves from enemies and a strong big well gaped mouth to fight with their enemies and procure food, developed air-sacs and extra accessory respiratory organ and variation in size, but inspite of all these, this line of evolution—being adapted only to fresh water habits could not bring much progress in evolution. A few of the fishes of this family acquired marine habits as *Aplocheilus melastigma, Firasfer, Zoarces* and *Lepidogaster* (Goodrich) which have functional pronephros in the adult (1). These fishes, on further evolution, might have gaven rise to amphibians.

This histological and metabolic evolution correctly place the fishes of Siluroideae family, whose origin is yet quiet obscure, very near to the lower vertebrates. If the evolution of the animals is studied in the light of the evolution of their metabolic processes, the different courses which the evolution tried will be made clearer because these metabolic changes were brought about in them so as to

enable them to adapt themselves to the environments and these in turn produced changes in their internal organs which carry out these activities, and these changes in the organs caused the development of various accessory structures for their protections as well as better functioning. Unfortunately, due to our lack of sufficient knowledge of these sudden metabolic processes of the animal we have concentrated more than necessary on the external forms of the different organs in our present classification and though this classification may prove helpful in classifying the animals in their systematic study, it does not clearly indicate the metabolic course which the system of animal followed during the process of evolution.

REFERENCES

1. Marshall, E. K., Jr. and Homer W. Smith, *Boil, Bull.* Vol., 59, 135, (1930).
2. Audige, I., *Arch de Zool Exper. at gen.*, 5 me Ser., 4, 275 (1910).
3. Rangarajan, K., *Current Sci.*, 20, 187 (1951).
4. Marshall, E. K., *Bull. Johns, Hopkin Hosp.* 45, 95, (1926).
5. Harmer, S. F. and Shiply, A. E. The Cambridge Natural History, The Macmillan Company, London, Vol. VII, 587 (1952).

STUDIES ON THE STRUCTURE AND BEHAVIOUR OF THE CHROMOSOMES OF GENOCEPHALUM ELONGATUM FAIRIN (COLEOPTERA, TENEBRIONIDAE)

By

UMA AGARWAL

Division of Microbiology, Central Drug Research Institute, Lucknow

[Received on 29th April, 1960]

The cytology of various species of the family Tenebrionidae of the order Coleoptera has been reported earlier by Stevens (1906), Nonidez (1914, 1915 and 1920), Guénin (1949, 1950, 1951*a*, *b*, 1953 and 1956), Smith (1952*a*, *b*, 1953) and Dutt (1953). According to Smith's chromosome list of Coleoptera (1953) the chromosomes of 36 species belonging to this family are known, but Makino (1956) in his new list has given the chromosome number of 37 species. Recently Guénin (1956) reported the chromosomes of *Caenoblaps nitida* Achüst. An account of three more species, *Alphitobius fagi* Panzer, *Tribolium ferrugineum* Fabricius and *Tribolium confusum* Duval has been given by Takenouchi (1957), of which *Tribolium confusum* has already been studied cytologically by Smith (1952 *a*, *b*) in considerable detail. Various interesting examples of multiple sex-chromosome mechanism have been reported in many species, belonging to this family by Nonidez (1914, 1915 and 1920), Guénin (1949, 1950, 1951*a*, *b*, 1953 and 1956) and Smith (1952 *a*, *b* and 1953).

The author in the present paper proposes to deal with the structure and behaviour of the chromosomes during mitosis and meiosis in *Genocephalum elongatum*.

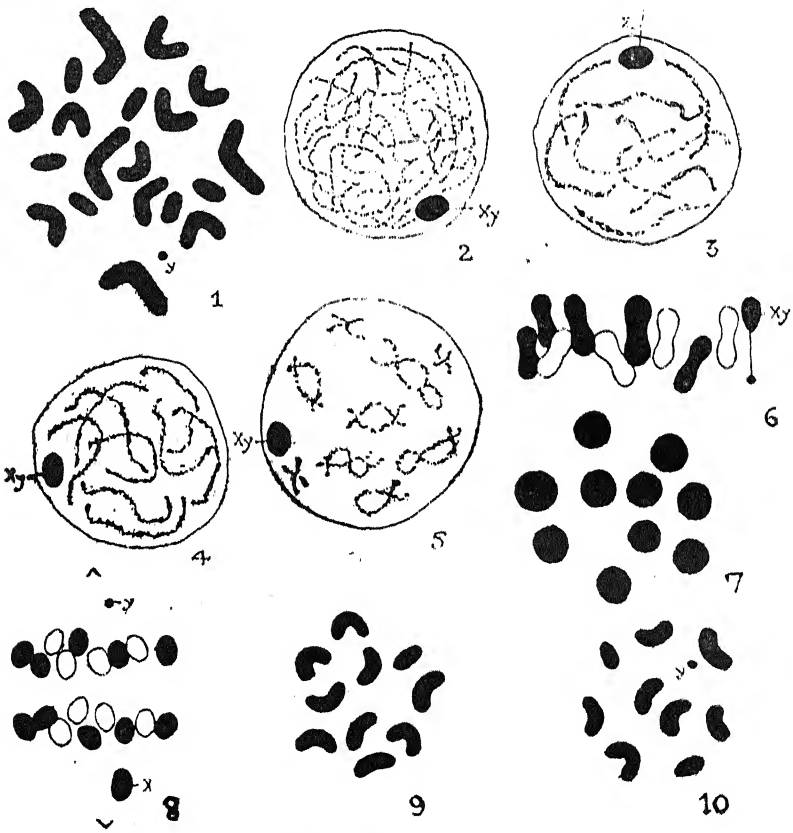
MATERIAL AND METHOD

The testes from the adult male specimens were fixed in Sanfelice and Corrosive sublimate acetic (sat) for 6-12 hours and sections stained with Newton's gentian violet and Feulgen's basic fuschin were examined. The diagrams were drawn with the help of camera lucida at a magnification 5000 X (approx.)

OBSERVATIONS

Spermatogonia—The spermatogonial metaphases show 20 chromosomes which can be classified as 19 large and one remarkably small in size (Fig. 1). Morphologically the large chromosomes can easily be sorted out into 13 metacentric and 6 acrocentric chromsomes. Among the metacentric group four are comparatively large in magnitude and rest are all medium-sized chromosomes. In an attempt to pair the homologous chromosomes according to their comparable shape and size it becomes evident that a minute, dot-shaped and one medium-sized V-shaped chromosome remain unpaired. The latter is most probably the X-chromosome. But the X-chromosome has no special feature to make it distinguishable from the autosomes at this stage, whereas the dot-shaped minute element without corresponding mate, is undoubtedly the y-chromosome which can be readily identified in the complement. The autosomes consist of two pairs of J-shaped, four pairs of V-shaped and three pairs of telomatic rod or kindney-shaped chromosomes.

Meiosis—During prophase the sex chromosomes X and y are found associated into a single, deeply-stained heteropycnotic mass lying excentrically in the nucleus, At leptotene (Fig. 2) the autosomes appear as fine, faintly-stained slender and granular threads. They become comparatively prominent at zygotene (Fig. 3) and on close examination the homologous chromosome threads are found lying close parallel to each other. The pachytene nucleus shows 10 elements of which 9 are autosomal bivalents and remainder is a sex chromosome mass. At late pachytene (Fig. 4) the autosomal threads become hairy in appearance, owing to the presence of Feulgen-positive, fine lateral processes or lampbrush fibres which stain faintly as compared to the chromosome threads. These fibres persist upto diplotene. The autosomal bivalents at diplotene contain 1-3 chiasmata each, depending on their length (Fig. 5). The autosomes become further condensed at diakinesis.



Figures 1—10. Chromosomes of *Genocephalum elongatum.*

1. Spermatogonial metaphase. 2. Leptotene stage of meiosis. 3. Zygotene. 4. Pachytene. 5. Diplotene. 6. First metaphase, side view. 7. The same, polar view. 8. Anaphase I. 9. Second metaphase, X-class. 10. The same, y-class.

The first metaphase contains 10 chromosomes consisting of 9 autosomal bivalents and an X-y pair (Fig. 6). The heteromorphic sex bivalent can be readily distinguished from the autosomes at this stage. The large X-chromosome remains connected with the minute y by means of a fine, Feulgen-positive thread. In the polar view (Fig. 7) all the chromosomes appear spherical, deeply-stained bodies, arranged more or less in a ring with few elements inside the ring. All the chiasmata present during diakinesis get fully terminalized at this stage.

At the first meiotic division the partners in bivalents separate normally. The separation of the sex chromosomes X and y precedes that of the autosomes and the former, therefore, reach the opposite pole earlier than the autosomes (Fig. 8). Two types of secondary spermatocytes are thus produced-one contains the X (Fig. 9) and the other the y (Fig. 10).

DISCUSSION

In *Genocephalum elongatum* the X and y sex chromosomes remain distinctly associated by a single point of contact at the first metaphase. Guénin (1950, 1951*a* and 1951*b*) in a series of papers reported the occurrence of similar association between the sex chromosomes, Smith (1950, 1951, 1952*a*, *b* and 1953) working on the cytology of certain tenebrionoids, incidently could not observe this type of association, and in the species he investigated, he found typical parachute X-y pair. He, therefore, casts serious doubts on the observations and the staining techniques employed by Guénin. My observations are, however, in agreement with those of Guénin. Such an association between the sex chromosomes has also been reported in other families by Stevens (1909), Asana, Makino and Niiyama (1942), Yosida (1944, 1946, and 1951) and Smith (1953).

SUMMARY

1. The chromosome number (2*n*) of the male of *Genocephalum elongatum* is 20 and the species has an XX:XY type of sex determination.

2. The sex chromosomes X and y are found in a condensed state throughout the prophase.

3. All the chiasmata present during diakinesis get fully terminalized at the first metaphase.

4. The sex chromosomes at the primary spermatocyte metaphase remain associated with each other by a single point of contact.

ACKNOWLEDGEMENT

This work was done in the Department of Zoology, Allahabad University, Allahabad. The author wishes to express her sincere gratitude to Professor M. D. L. Srivastava for his keen interest and kind guidance.

REFERENCES

Asana, J. J., Makino, S., and Niiyama, H., 1942, *Cytologia*. **12**, 187-205.

Dutt, M. K., 1953, *Current Science*, **22**, 278.

Guénin, H. A., 1949, *Rev. Suisse Zool.*, **56**, 336.

———— 1950, *Genetica*. **25**, 157-182.

———— 1951a, *Bull. Soc. vaud. Sc. Nat.*, **65**, 7-18.

———— 1951b, *Rev. Suisse Zool.*, **58**, 471-475.

———— 1953, *Rev. Suisse Zool.*, **60**, 462-466.

———— 1956, *Rev. Suisse Zool.*, **63**, 298-303.

Makino, S., 1956, A review of the chromosome numbers in animals. Hokuryukan, Tokyo.

Nonidez, J.F., 1914, *Trad Mus. Cienc. Natur, Madrid Ser. Zool.*, **18**, 5-97.

———— 1915, *Mem. R. Soc. esp. His. nat.*, **10**, 149-190.

———— 1920, *Jour. Morph.*, **34**, 69-117.

Smith, S. G., 1950, *Canad. Entom.*, **82**, 58-68.

———— 1951, *Genetica*, **25**, 522-524.

———— 1952a. *Chromosoma*, **4**, 585-610.

———— 1952b, *Jour. Morph.*, **91**, 325-364.

———— 1953, *Heredity*, 7, 31-48.

Stevens, N. M., 1906, *Carneg. Inst. Wash. Publ.* no. **36**, 33-74.

———— 1909, *J. Exp. Zool.*, **6**, 101-121.

Takenouchi, Y., 1957, *Jour. Fac. Sci Hakkaido Univ.*, Ser. VI, Zool. **13**, 315-319.

Yosida, T. 1944, *Jap. J. Genet.*, **20**, 107-115.

———— 1946, *Seibutu.*, **1**, 218-223.

———— 1951, *La Kromosomo.* **9**, (Makino, S. 1951. An Atlas of the Chromosome Numbers in Animals. The Iowa State College Press, Ames.)

CHROMOSOME COMPLEMENT AND MEIOSIS IN TWO SPECIES OF BRUCHIDAE (COLEOPTERA)

UMA AGARWAL

Division of Microbiology, Central Drug Research Institute, Lucknow

[Received on 17th June, 1960]

There seems to be a paucity of information regarding the chromosomes of the family Bruchidae. Only three species belonging to this family were cytologically known previously (Brauer, 1928 ; Minouchi, 1935 and Bushnell, 1936). Recently Takenouchi (1955) reported the chromosomes of three more species *Bruchus pisorum*, *Bruchus rufimanus* and *Callosobruchus chinensis*. Of late years, the present author has been engaged in the cytological study of Coleoptera and has had an opportunity to investigate the chromosomes of two species of Bruchidae *Bruchus analis* Fabr. and *Pachymerus chinensis* L., the results of which form the subject matter of the present paper.

MATERIAL AND METHOD

All the specimens for the present study were collected from infested grains available in local markets throughout the year. The gonads were fixed in Sanfelice and Corrosive sublimate acetic (sat.) for 6-12 hours. Sections stained with Newton's gentian violet and Feulgen's basic fuschin were examined. The diagrams were drawn with the help of camera lucida at a magnification 5000 × (approx).

OBSERVATIONS

1. *Bruchus analis* (Figs.) 1-10.

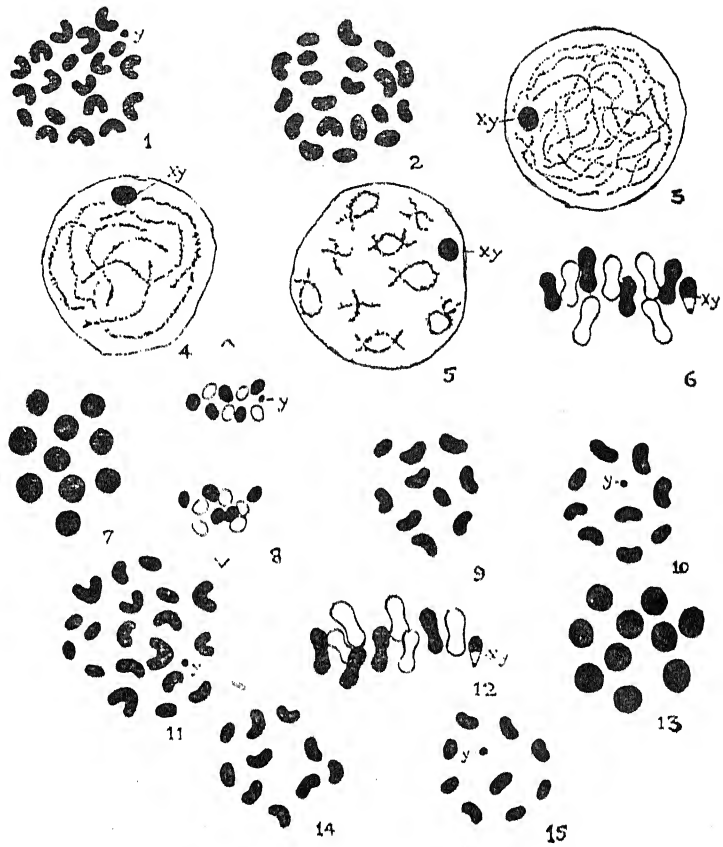
Spermatogonia.—The chromosome complement at spermatogonial metaphase shows 20 chromosomes (Fig. 1). Two pairs of rods and a small dot—shaped chromosome, stand out distinct in the complement, while the remaining fifteen are apparently metacentric in nature. The minute spherical element is not present in oogonial metaphase (Fig. 2) and is, therefore, obviously the y-chromosome. The X is one of the V-shaped chromosomes but it remains indistinguishable from the autosomes at this stage. The sex chromosomes do not show any heteropycnosis.

Meiosis.—During prophase the sex chromosomes (X and y) are found associated into a single deeply—stained heteropycnotic mass which always lies near the periphery. The autosomes at leptotene (Fig. 3) appear as fine, faintly—stained granular threads running into each other. The late zygotene or early pachytene nucleus shows 10 elements consisting 9 autosomal threads and a heteropycnotic sex chromosome mass. At late pachytene (Fig. 4) the autosomal threads appear hairy due to the presence of fine, faintly—stained, Feulgen positive lampbrush fibres which presist upto early diplotene. At diplotene (Fig. 5) the autosomes have 1-2 chiasmata each depending on their length. The diplotene is succeeded by the diakinesis during which the autosomal bivalents become further condensed.

The primary spermatocyte metaphase (Fig. 6) consists of 10 bivalents, one of which is a heteromorphic X-y bivalent of a characteristic parachute form. As seen in the polar view all the chromosomes appear spherical deeply-stained bodies (Fig. 7). As the result of the first division (Fig. 8), the two kinds of the secondary spermatocytes are produced, each having 10 chromosomes ; one contains the X element (Fig. 9), while the other the y (Fig. 10).

2. *Pachymerus chinensis.* (Fig. 11-15).

Spermatogonia.—Every spermatogonial metaphase shows 20 chromosomes classifiable into two pairs of larger V-shaped chromosomes, a minute spherical one and the remaining fifteen medium-sized chromosomes of different shape—oval, rod, kidney and V-shaped (Fig. 11). By pairing the homologous mates according to their



Figs. 1—10. Chromosomes of *Bruchus analis.*

1. Spermatogonial metaphase. 2. Oogonial metaphase. 3. Leptotene stage of meiosis. 4. Pachytene. 5. Diplotene 6. First metaphase. (side view). 7. First metaphase (polar view). 8. Anaphase I. 9. Second metaphase X-class. 10. The same, y-class.

Figs. 11—15. Chromosomes of *Pachymerus chinensis.*

11. Spermatogonial metaphase. 12-13. First metaphases.

14. Second metaphases, X-class. 15. The same, y-class.

comparable shape and size it becomes evident that a smaller rod-shaped represents the X-chromosome and the minute spherical element is the y.

Meiosis.—The prophase stage is very much similar to that of *Bruchus analis.* The first metaphase shows 10 chromosomes comprising 9 autosomal bivalents and an X-y pair (Figs. 12-13). The X-y complex is clearly recognizable by its parachute

form in the side view. At anaphase I the X and y chromosomes segregate migrating to the opposite poles along with the autosomes. The first division results into two types of secondary spermatocytes—one with nine autosomes plus an X and the other with the same number of autosomes plus a y (Figs. 14-15).

SUMMARY

The chromosomes of two species of the family Bruchidae (Coleoptera) have been studied with regard to the morphology of chromosomes. Both the species have XX : XY type of sex-determining mechanism and the chromosome formula 9AA + Xyp. The sex chromosomes are found in a condensed state throughout the prophase stage. The results (chromosome number and sex-determining mechanism) are summarized in Tabel 1. in comparison with those reported for other species by previous authors.

TABLE 1

Species	Chromosome number 2 n	Chromosome number n	Sex-chr.	Author
Zabrotes subfasciatus Boheman	26 s	13, 14 (I) 12, 13, 14 (II)	X-O (1-super num. in)	Minouchi '35
Acanthoscelides obtectus Say	20 m	10 (I)		Bushnell '36
Bruchus quadrimaculatus	19 s	10 (I) 9, 10 (II)	X-O	Brauer '28
Bruchus pisorum Linné	22 s	11 (I, II)	X-Y	Takenouchi '55
Bruchus rufimanus Boheman	37 s	19 (I) 18, 19 (II)	X-O	,, ,,
Callosobruchus chinensis Linné (*Bruchus chinensis* Linné)	19 s	10 (I) 9, 10 (II)	X-O	,, ,,
Bruchus analis Fabr.	20 s	10 (I, II)	X-Y	This paper
,, ,,	20 o		X-X	,,
Pachymerus chinensis L.	20 s	10 (I, II)	X-Y	,,

s : spermatogonium, m : somatic mitosis, (I) : primary spermatocyte, (II) : secondary spermatocyte, o : oogonium.

ACKNOWLEDGMENTS

The work was carried out at the Department of Zoology, Allahabad University, Allahabad. The author is deeply grateful to Proof. M. D. L. Srivastava for his

kind guidance and encouragement. Thanks are also due to Dr. A. P. Kapur, Zoological Survey of India for identification of the material.

REFERENCES

Brauer, A. 1928, Spermatogenesis of *Bruchus quadrimaculatus* (Coleoptera : Bruchidae), *Jour. Morph.*, 46 : 217–240.

Bushnell, R. J., 1936, The development and metamorphosis of the mid-intestinal epithelium of *Acanthoscelides obtectus Say*. *Jour. Morph.*, **60** : 221-241.

Minouchi, O., 1935, Über die Chromosomen des Bohnenkäfers *Zabrotes* subfasciatus Boh. (Bruchidae), *Zeits. Zellf. mikr. Anat.*, **23** : 361-374.

Takenouchi, Y., 1955, A short note on the chromosomes in three species of the Bruchidae (Coleoptera), *Jap. Jour. Genet.*, **30** : 7-9.

THE CHROMOSOME CYTOLOGY OF *CHLAENIUS PANAGAEOIDES* CHAUD (COLEOPTERA)

By

UMA AGARWAL*

Department of Zoology, University of Allahabad, Allahabad

[Received on 28th June, 1960]

ABSTRACT

The diploid chromosome number of the male of *Chlaenius panagaeoides* is 35, but all the species belonging to this genus studied so far, except one which possesses 9AA+Xy, show either 34 or 37 chromosomes. *C. panagaeoides* has an XX: XO mode of sex-determining mechanism. The X-chromosome can be readily identified during early meiotic stages due to its highly condensed nature. The diakinesis seems to be very short-lived.

INTRODUCTION

Cytology of various species of the family Carabidae of the order Coleoptera has been reported earlier by Stevens (1906), Asana *et al* (1942), Yosida (1951) and Smith (1953). Reference to the Smith's chromosome list of Coleoptera (1953) the chromosomes have so far been reported in 24 species belonging to this family. Recently, the present author has been engaged in the cytological study of Coleoptera, and has had an opportunity to investigate the chromosomes of two species of Carabidae *Pheropsophus bimaculatus*, 17AA+X (Agarwal, 1960) and *Chlaenius panagaeoides*, the results of which are to be presented in this paper. The cytological knowledge of Indian Coleoptera (beetles) is very meagre. Asana *et al* (1942) and and Bose (1948) have recorded a few species.

The work was carried out at the Department of Zoology, University of Allahabad, Allahabad. The author wishes to express her grateful thanks to Prof. M. D. L. Srivastava for his kind guidance and to Dr. A. P. Kapur, Zoological Survey of India, Calcutta for the identification of the material. The financial support by the University of Allahabad is also thankfully acknowledged.

MATERIAL AND METHOD

The testes from the adult male specimens were fixed for 6-12 hours in Sanfelice, and Corrosive sublimate acetic (sat). Sections stained with Heidenhain's haematoxylin, Newton's gentian violet and Feulgen's basic fuschin were examined. The camera lucida sketches reproduced here have been drawn at a magnification of 5000 X (approx).

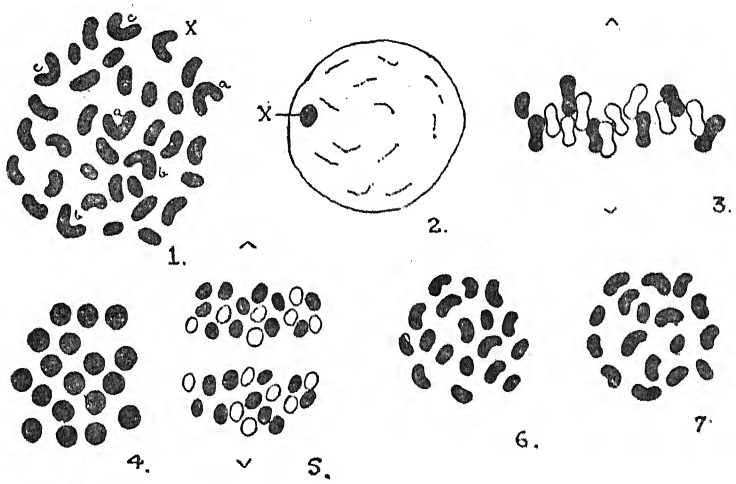
OBSERVATIONS

The chromosome complement at every spermatogonial metaphase show 35 chromosomes which cannot be put into different size-classes as they are more or less similar in size (Fig. 1.). It is, however, possible to identify seven V-shaped metacentric chromsomes in the complement, while the remaining 14 pairs of chromosomes are rod or kidney shaped, In the metacentric group six chromosomes form three homologous pairs of autosomes (*aa*, *bb*, *cc*) and the unpaired one is probably

* Present address : C-3/2, River Bank Colony, Lucknow.

the X-chromosome. But the latter has no special characteristic feature distinguishable from the autosomes at this stage.

Meiosis—During prophase the X-chromosome can easily be distinguished from the autosomes by virtue of its highly condensed nature (Fig. 2). Following diakinesis which is very short-lived the chromosomes assume at first metaphase, the form of simple rods with median constriction marking the position of completely terminalized chiasma (Fig. 3.). The first metaphase polar view (Fig. 4) exhibits 18 deeply-stained spehrical bodies of which 17 are autosomal bivalents and the remaining one is the univalent X-chromosome. At anaphase I (Fig. 5) the partners in autosomal bivalents separate normally and the X-chromosome goes intact without dividing to one of the poles of the spindle along with the autosomes.



Figs. 1—7. Chromosomes of *Chlaenius panagaeoides*.

1. Spermatogonial metaphase. 2. Early prophase, showing the heteropycnotic sex or X-chromo-some. Fig. 3-4. First metaphases. Fig. 5. Anaphase I. Fig. 6. Second metaphase, with X-chromosome. Fig. 7. The same, without X-chromosome.

As the result of the first division, two numerical types of secondary spermatocytes are formed, the one having 17 autosomes plus the X-chromosome (Fig. 6), while the other contains 17 autosomes only (Fig. 7).

CONCLUDING REMARKS

The spermatogonial complement of *Chlaenius panagaeoides* (investigated by the author) is observed to have 35 chromosomes *i.e.* two less than what is found in majority of the species of this genus. Two species belonging to the same genus have been worked out by Stevens (1906). In *C. aestivus* she observed 34 chromosomes (32 autosomes+X+y), whereas in *C. pennsylvanicus* she reported only 20 chromosomes (18 autosomes + X + y) in the heterogamatic sex. Later on Yosida (1951) and Smith (1953) reported the chromosome number of three more species of the genus *Chlaenius*, *C. pallipes*, *C. tricolor* and *C. laticollis* as 37 (36 autosomes + X). Such a striking difference in chromosomes between species belonging to one genus has not been observed elsewhere in the order. The carabids in general, exhibit high chromosome numbers except *C. pennsylvanicus*. The frequency distribution of

the chromosome number in the family Carabidae is represented diagrammatically in Fig. 8 which is based on 26 species.

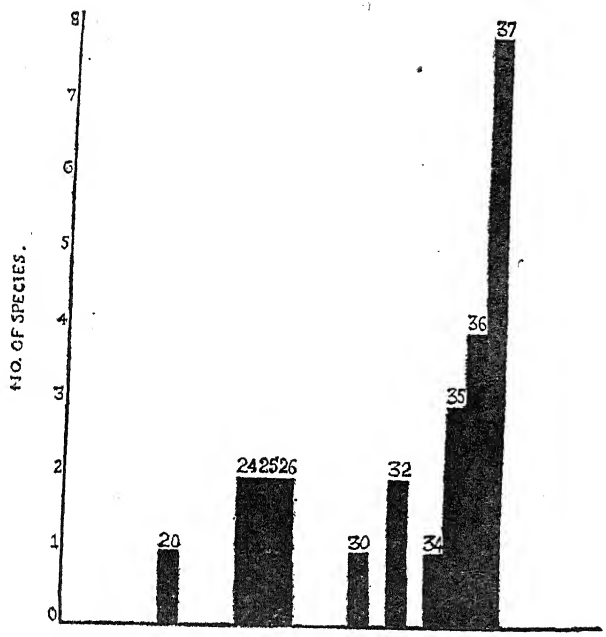


Fig. 8. Histogram showing the frequency distribution of chromosome number (diploid) in the family Carabidae.

Fig. 8, clearly shows that the chromosome number 37 gives the highest peak in this family while the rest of the numbers are represented by comparatively low frequencies. But further investigations of other species may change the shape of this frequency histogram, either by bridging the existing gap or extending the known range of variation, because most of the genera are known so far by only few species each. The increase in the number of chromosomes is probably due to the fragmentation or separation of the arms of previously metacentric V-shaped chromosomes, as has already been suggested by Smith (1950). Although little can be said, *C. panagaeoides* besides lacking the y, has diverged further presumably by autosomal fragmentation and may have evolved from *C. aestivus* through the addition of a pair of autosomes (by fragmentation) and the loss of the minute y-chromosome which is almost near the limit of visibility.

SUMMARY

The diploid complement of *C. panagaeoides* is 35, and the mode of sex determination is XX : XO type. The X-chromosome which remains indistinguishable from the autosomes at the spermatogonial divisions, can be readily identified during prophase due to its highly heteropycnotic nature. At anaphase I the X goes undivided to one of the poles of the spindle synchronously with the autosomes.

REFERENCES

Agarwal, U. 1960, Chromosome complement and meiosis in *Pheropsophus bima culatus* (Coleoptera, Carabidae), *Curr. Sci.*, 29, 284-285.

Asana, J. J., Makino, S., and Niiyama, H., 1942, A chromosome survey of some Indian insects—IV, *Cytologia*, 12 : 187-205.

Bose, I. 1948, The association of non-homologous chromosomes in the spermatogenesis of the lady bird bettle, *Epilachna vigintioctopunctata* (Fab.), *Proc. Zool. Soc. Bengal.*, 1 : 131-139.

Smith, S. G. 1953, Chromosome numbers of Coleoptera, *Heredity.*, 7 : 31-48.

Stevens, N. M. 1906, Studies in spermatogenesis, II. A comparatative study of the hetero-chromosomes in certain species of Coleoptera, Hemiptera and Lepidoptera with especial reference to sex determination, *Carneg. Inst. Wash., Pub.* no. 36, Part II, 33-74.

Yosida, T. 1951, La Kromosomo 9, (quoted from Smith 1953).

ON THE RESPONSE OF DIFFERENT AGES OF *BRUCHUS ANALIS* FABRICIUS AND *CORCYRA CEPHALONICA* STAINT. TO CARBONBISULPHIDE

By

SNEHAMOY CHATTERJI

Division of Entomology, Indian Agriculture Research Institute, New Delhi-12

[Received on 20th November, 1959]

Sun (1947) studied the effect of carbonbisulphide on different ages of *Tribolium oonfusum* Jacq. Dev. and found that the susceptibility of eggs increased consistently with age from 1 to 7 days. He found that the susceptibility of the larvae of various ages was different from that of the eggs. Their susceptibility increased from 1 day to 7 days after which there was no appreciable change upto 14th day. He also observed further that after 14th day their susceptibility decreased as the age increased. Broadly speaking *T. confusum* adults were found to be more susceptible to the fumigant than the younger ones. He, however, found 7 days old adults to be more resistant than 4 days old adults.

Laboratory experiments were carried out on the response of different ages of *Bruchus analis* Fabr. namely, 1 day, 6 days, 12 days old grubs; 1 day, 3 days and 5 days old pupae and 1 day, 6 days and 12 days old adults to carbonbisulphide. All the above ages were within the seeds of *Phaseolus aureus* (Mung). One day, 16 days and 32 days old caterpillars; 1 day and 7 days old pupae and 1 day and 5 days old adults of *Corcyra cephalonica* Staint. were also included in the experiment. The cultures of the above test insects were maintained at a temperature of 27°C ± 1°C and 65% relative hnmidity. In order to obtain the various ages of different stages of the above, 100 adults of each of these pests were introduced in several glass jars (8" high and 6" in diameter) containing 1000 grams of 'mung' and crushed 'jowar' (*Adropogon sorghum*) respectively. The moths were selected from cultures at 20°C. This precaution was taken because it was observed that unsuccessful pairing occurred in the case of moths reared at 27°C or at higher temperatures.

This observation was also made by Norris (1932) in the case of the *Ephestia kuehniella* Zell. Just after 12 hours both the 'mung' seeds as well the crushed "jowar" were sieved out and the pests introduced in another set of jars containing the same weight of these two grains. Each lot of the pest was used only twice. In those cases where after the first sieving the pests were found inactive or sometimes dead they were removed and equal number of fresh specimens replaced. In the case of *Bruchus analis* the eggs are glued on to the pulse so that the 'mung' escaped through the sieve with the eggs glued on to them. In the case of *C. cephalonica* the eggs are laid loose. Therefore, along with the grains the eggs also passed through the sieves leaving only the adults on the sieves. This process was repeated and at least four cultures of these two pests were made every day so that there was always a continuous supply of the various stages of the two test insects and their ages for experimental purposes.

The fumigation with carbonbisulphide was done in air tight, cylindrical iron bins at room temperature and humidity varying from 71°F to 100°F and

25% to 65% respectively. One hundred infested seeds of 'mung' having the respective immature stages of *B. analis* or the naked stages of *C. cephalonica* were taken in cylindrical wire-gauze tubes (3" high and 2" in diameter) closed at the top with cork. Four different concentrations were tried namely 1 lb, 2 lbs, 3 lbs and 5 lbs per 1000 C. ft of space. The period of exposure was 24 hours in every case. The wire-gauze cages containing test insects of a particular age of *C. cephalonica* were corked properly and hung inside the fumigatorium by means of twine thread along with the cages containing the infested seeds having different ages of *B. analis* in them so that they reached exactly all round the centre of the bin. There were 3 replications in each treatment with each age of the pest. The required quantity of the liquid fumigant in cubic centimeters was calculated by the formula given below.

$$\frac{B \times A \times 454}{1000 \times \text{sp. gravity of the fumigant}}$$

Where A = cubic space in ft. to be fumigated.

B = dosage in lbs per 1000 c. ft. of space.

Sp. gravity of carbonbisulphide = 1·261.

The measured liquid fumigant was poured directly from a burette inside an iron bin through the central hole of its lid. Immediatelly after pouring the fumigant the iron-screw cap of the central hole of the lid was closed airtight. The liquid fumigant thus poured was received on a piece of cotton wool at the topmost part of the bin. The percentage of mortality in the case of *B. analis* was calculated by recording the total number of beetles that emerged from the infested seeds which were fumigated. In the case of *C. cephalonica* the percentage of mortality was calculated by the number of dead insects in their respective agess. 'Checsk' or control lots were also kept in 3 replicates to compare with the natural mortality during the observation period. The treated and the control lots in the case of *B. analis* were observed for 8 days from the first day of emergence of adult from the seeds having immature stages. The bruchid adults were however observed for 3 days, while on the other hand in *C. cephalonica* the observations for mortality were taken upto 6 days in the case of caterpillars, till the emergence or otherwise in the case of pupae and for 3 days in the case of adults. The procedure for the control lots was similar except that they were not treated with any fumigant. Finally, the adjusted mortality was calculated by the following formula.

$$\frac{100\,(a-b)}{100-b}$$

Where a = % mortality in treatd lots and

b = % mortality in control lots.

From the observations recorded (vide Tables No. 1 and 2), it can be concluded that the resistance of the grubs of *B. analis* and the caterpillars of *C. cephalonica* increases with the increase in age. The pupa of *B. analis* on the whole becomes more resistant with the increase in age although the degree of resistance in 1 day old pupa and 3 days old pupa does not differ significantly. The pupa of *C. cephalonica* also increases in resistance with the increase in age. The resistance of the adults of *B. analis* gets markedly reduced with the advancement in age as in the case of the adults of *C. cephalonica*. The mortality figures of 1 day and 5 days old adults of the latter, however, do not differ significantly between themselves. The pupa of both *B. analis* and *C. cephalonica* have been found to be the most resistant in comparison with their respective grubs and adults of various ages. In degree of resistance the

TABLE 1

Average % of abjusted mortality of different ages of *Bruchus analis*, Fabr. to Carbonbisulpide

Serial No.	Ages of *Bruchus analis*	Avg. adjusted mortality at 1 lb. level	Avg. adjusted mortality at 2 lbs. level	Avg. adjusted mortality at 3 lbs. level	Avg. adjusted mortality at 5 lbs. level
1	1 day old grub	9·6 %	22·6 %	49·6 %	84·3 %
2	6 days old grub	7·6 %	17·6 %	44·8 %	72·6 %
3	12 days old grub	5·0 %	14·0 %	35·4 %	67·3 %
4	1 day old pupa	5·3 %	12·3 %	34·0 %	63·0 %
5	3 days old pupa	5·0 %	12·3 %	35·0 %	61·0 %
6	5 days old pupa	1·6 %	7·6 %	23·0 %	56·6 %
7	1 day old adults	42·6 %	67·6 %	81·3 %	100 %
8	6 days old adults	66·0 %	79·6 %	95·3 %	100 %
9	9 days old adults	73·2 %	96·3 %	100 %	100 %

TABLE 2

Average % of adjusted mortality of different ages of *Corcyra cephalonica*, Staint to Carbonbisulphide

Serirl No.	Ages of *Corcyra cephalonica*	Avg. adjusted mortality at 1 lb. level	Avg. adjusted mortality at 2 lbs. level	Avg. adjusted mortality at 3 lbs. level	Avg. adjusted mortality at 5 lbs. level
1	1 day old caterpillars	13·0 %	33·3 %	57·1 %	96·8 %
2	16 days old caterpillars	11·3 %	29·3 %	51·3 %	88·1 %
3	32 days old caterpillars	6·0 %	21·3 %	40·8 %	77·0%
4	1 day old pupae	1·3 %	9·6 %	27·6 %	61·3 %
5	7 days old pupae	Nil	6·0 %	24·6 %	55·6 %
6	1 day old adults	62·0 %	85·3 %	100 %	100 %
7	5 days old adult	63·2 %	87·8 %	100 %	100 %

larval stages of both the above pests come next to pupa. The adults are, however, most susceptible.

ACKNOWLEDGEMENT

The author is grateful to Dr. E. S. Narayanan, M.A. Ph.D (Lond.), D.I.C. F.R.E.S., F.E.S.I., F.A.Sc., F.N.I., Head of the Division of Entomology, for the help rendered by him in various ways.

REFERENCES

1. Norris, M. J., 1932, Contributions towards the study of insect fertility I. The structure and operation of the reproductive organs of the genera *Ephestia* and *Plodia.*, *Proc. Zool. Soc.*, London: 595—611 (I); 903 —934 (II).

2. Sun, Y. P., 1947, An analysis of some impotent factors affecting the results of fumigation tests on insects, *Minnesota Exptl. Station. Technical Bullentin* No. 177.

SECRETION OF PROTOPECTINASE ENZYME BY *FUSARIUM ORTHOCERAS* APP. & WR. VAR. *CICERI* PADWICK ON NATURAL MEDIA

By

M. N. GUPTA

*Botany Department, Agra College, Agra.**

[Received on 12th July, 1960]

INTRODUCTION

Fusarium orthoceras App. & Wr. Var. *ciceri* Padwick is an important pathogen in India causing vascular wilt of gram, *Cicer arietinum* (Padwick, 1940; Subramanian, 1954; Chauhan, 1957).

In recent years the importance of the various pectic enzymes secreted by pathogens in the production of the symptoms of wilt has been emphasized (Gothoskar *et al*, 1955 and Winstead and Walker, 1954). In the present paper the secretion of the intra- and extra-cellular protopectinase enzymes by *F. orthoceras* var. *ciceri* on various natural media has been reported.

MATERIALS AND METHODS

The fungus used for the present investigation was isolated from the wilted gram plants collected from the cultivated fields of Agra and the stock culture was maintained on potato-dextrose-agar medium.

Several fruits and other parts of different plants like seeds, rhizome, tuber, etc. were used for the preparation of media by the following different methods :—

(*a*) *Decoction media*—200 gm. of plant material was boiled for an hour in distilled water, filtered through fine muslin and the filtrate made upto 1 litre. Decoctions of seeds and seedlings (2·5%—5%) were also prepared in a similar manner.

(*b*) *Minced media*—200 gm. of potato tuber, tomato and gram seedlings were minced separately in presence of 1 litre of distilled water in a Waring Blendor. The liquid was used after filtration through muslin.

(*c*) *Extract media*—40 gm. of healthy potato tubers were frozen at-4°C for 48 hours, brought to room temperature by keeping in a trough of water, sap squeezed by hand and made upto 200 ml.

(*d*) *Pulp media*—Tomato pulp, as sold in the market, was diluted to five times and used as such as medium.

(*e*) *Slices*—About 5 mm. thick slices of potato and tomato were cut and autoclaved in Petridishes before inoculation.

Besides these media fresh fruits and other parts of the plants were also inoculated by the method described by Bhargava and Gupta (1957).

*The work was done in the Botany Department, Th. D. S. B. Government College, Naini Tal.

Only 15 ml. of liquid medium was taken in each flat bottle of 12 fluid oz. capacity. The sterilization was done by autoclaving at 15 lbs. pressure for 15 minutes. Each bottle and petridish was inoculated with five small bits of the inoculum from a 5-6 days old culture which had good sporulation. The bottles were then shaked in order to spread the inoculum uniformly and were stacked along with the Petridishes in an incubator at 25°C. After the required incubation period the fungal mat was removed and the clear liquid was used as such for testing the activity of extracellular protopectinase enzyme. In the case of potato and tomato slices the sap was squeezed from the infected portions, cleared by centrifuging and tested for the enzyme activity.

For testing the intra-cellular enzyme the mycelium mat was crushed in presence of carborandum powder and 10 ml. of distilled water was added to it. The liquid was centrifuzed and used for test.

The enzyme activity was estimated at room temperature (22°C-27°C) by the potato disc method of Brown (1915) and has been expressed in time taken to macerate (MT) 3 potato discs of 8 mm. diameter and 0·5 mm. thickness when kept in 5 ml. enzyme preparation.

EXPERIMENTAL

Extra-cellular enzyme—The activity of protopectinase enzyme was examined on a number of decoction media but only lemon albedo, brinjal, carrot, potato, tomato and lucerne media were found to be most satisfactory (Table I). However, best activity of the enzyme was found to be on lemon decoction after five days incubation. In another set of experiments 0·25% ammonium nitrate was added to the decoction media, but no improvement in the activity of secreted enzyme was noted.

TABLE 1

Enzyme secretion on different decoction media

Medium	Initial pH.	3 days incubation		5 days incubation		7 days incubation	
		Final pH.	MT in hours	Final pH.	MT in hours	Final pH.	MT in hours
Ginger	6·8	8·4	10-12	8·4	5½-6	8·6	>23
Brinjal	5·3	7·9	18-20	7·9	2	8·2	5
Beet root	6·5	5·8	4½	5·1	3½-3¾	6·0	5-5½
Lemon albedo	4·5	8·2	2-2½	8·4	1	8·6	1½-2
Banana	5·8	8·5	>22	8·5	>23	8·5	>23
Carrot	6·3	6·7	12-14	7·1	2	7·5	3-3½
Potato	6·4	8·1	2-2¼	8·3	5¼-5½	8·4	>23
Tomato	4·5	6·1	1½-2	8·2	2-2½	8·3	2-2½
Bean (Green pods)	5·9	8·2	>22	8·5	5·5¼	8·5	20-22
Lucerne seeds	6·9	7·7	1½	8·1	4	8·2	18-20
Gram seeds	6·7	7·8	8-10	8·3	4	8·3	4¾-5
Gram seedlings	5·6	7·9	23	8·2	20-22	8·2	>23

When the enzyme was obtained on natural media other than decoctions, the activity of the protopectinase did not improve in potato and tomato (Table II). However, minced gram seedlings gave better results than decoction media of gram seeds and gram seedlings.

In general the pH increased with the incubation period.

TABLE II

Enzyme secretion on various natural media.

Medium	Initial pH.	3 days incubation		5 days incubation		7 days incubation	
		Final pH.	MT in hours	Final pH	MT in hours	Final pH	MT in hours
Potato extract	5·5	7·5	>23	8·2	>23	8·2	>23
Potato minced	5·3	7·0	16-18	8·6	8-10	8·9	>23
Potato slices	–	7·6	2½-3	–*	–	–	–
Tomato pulp	4·3	5·5	5	5·7	8-10	6·5	10-12
Tomato minced	4·3	7·1	4½-5	8·1	8-10	8·5	22
Tomato slices	–	5·2	2½-3	–*	–	–	–
Gram seedling minced	5·6	7·3	2½-3	8·8	4	9·0	5

*Complete rot of the slices was caused within 3 days.

Fresh fruits and other parts of plants were inoculated and it was found that in ginger, carrot, beet root and brinjal there was very little growth of the fungus and no rot of the tissue took place even after an incubation period of 11 days. On tomato and bean pods, however, the fungus showed luxurient growth and on the 7th day the percentage of rot was 56 and 77·7 respectively. Complete rot (100%) was observed on the 11th day in both the cases, but there was no production of active protopectinase enzyme on tomato while in the case of bean pods the enzyme was comparatively very active. An equally active enzyme was produced on banana in which there was only 18·5% rot on the 11th day. The results are given in Table III.

TABLE III

Enzyme secretion on fresh plant tissues.

Fresh plant tissue	7 days incubation			11 days incubation		
	% of rot.	pH of extract	MT in hours	% of rot.	pH of extract	MT in hours
Carrot	–	–	–	–	–	–
Brinjal	–	–	–	–	–	–
Bean (Green pods)	77·7	7·6	1¾-2	100	8·6	1-1¼
Banana	12	7·3	3½-3¾	18·5	5·5	1-1¼
Ginger	–	–	–	–	–	–
Beet root	–	–	–	–	–	–
Tomato	55·9	4·8	6-8	100	5·3	5-6
Potato	17·8	7·4	2-2¼	27	7·1	1¼-1½

Intra-cellular enzyme—The production of intra-cellular protopectinase enxyme was also tested on various media. It was found that only in the decoctions of carrot and brinjal the fungus produced intra-cellular enzyme but of low activity while in all the other cases the enzyme activity was almost nil. The results are shown in tables IV and V. Like the extra-cellular enzyme, intra-cellular enzyme of better activity was not obtained when 0·25% ammonium nitrate was added to the decoctions.

TABLE IV

Intra-cellular enzyme on different decoction media.

Medium	3 days incubation. MT in hours	5 days incubation. MT in hours	7 days incubation. MT in hours
Ginger	>23	>23	>23
Beet root	19	>23	>23
Carrot	>23	3	>23
Lemon albedo	18	18	>23
Banana	17	>23	>23
Brinjal	>23	2½-2¾	>23
Tomato	18	16	20
Lucerne seeds	20	>23	>23
Gram seeds	>23	>23	>23
Potato	>23	>23	>23
Bean (Green pods)	>23	>23	>23
Gram Seedlings	>23	>23	>28

In all the cases pH was about 7.

TABLE V

Intra-cellular enzyme on varions natural media.

Medium	3 days incubation. MT in hours	5 days incubation. MT in hours	7 days incubation. MT in hours
Potato extract	>23	>23	>23
Potato minced	>23	>23	>23
Potato slices	22	–*	–
Tomato pulp	>23	>23	12-14
Tomato minced	>23	18-20	>23
Tomato slices	>23	–*	–
Gram seedlings minced	>23	18-20	>23

*Complete rot of the slices was caused within 3 days.

SUMMARY

Fusarium orthoceras var. ciceri secretes fairly active protopectinase enzyme on decoctions of lemon albedo, brinjal, carrot, potato, tomato and lucerne.

The pathogen was also found to cause rot when potato tubers and fruits of banana, tomato and bean (green pods) were inoculated. Protopectinase enzyme was found to be present in the sap of infected tissues.

The fungus produced very little intra-cellular enzyme on the different media used.

ACKNOWLEDGEMENT

The author is grateful to Dr. S. C. Gupta, Professor of Botany, Th. D. S. B. Government College, Naini Tal, for suggesting the problem and guiding the work.

REFERENCES

1. Bhargava, K. S. & Gupta, S. C. 1957, Market diseases of fruits and vegetables in Kumaon. II. Rhizopus rot of plums (*Prunus communis*), *Hort. Advan.* I: 65-67.

2. Brown, W. 1915, Studies in the Physiology of Parasitism. I. The action of *Botrytis cinerea. Ann. Bot. Lond.*, **29**, 313-348.

3. Chauhan, S. K. 1957, Disease intensity and crop-loss estimates in the wilt of Gram, *Proc. 44th Indian Sci. Cong.* **Part III**, 217.

4. Gothoskar, S. S., Scheffer, R. P., Walker, J. C. & Stahmann, M. A. 1955, The role of enzymes in the development of Fusarium wilt of tomato, *Phytopathology*, **45**, 381-387.

5. Padwick, G. W. 1940, The genus *Fusarium*. III. A critical study of the genus causing wilt of gram (*Cicer arietinum* L.) and of the related species in the sub-section Orthocera with special relation to the variability of key characters, *Indian J. Agri. Sci.*, **10**, 241-284.

6. Subramanian, C. V., 1954. Studies on south Indian Fusaria. III. Fusaria isolated from some crop plants, *J. Madras Univ.*, B. **24**, 21-46.

7. Windead, N. N. & Walker, J. C., 1954, Production of Vascular browning by metabolites from several pathogens, *Phytopathology*, **44**: 153.

STUDIES ON MUCORALES III.

PIPTOCEPHALIS DE-BARYANA SP. NOV.

By

B. S. MEHROTRA

Botany Department, University of Allahabad, Allahabad.

[Received on 20th June, 1960]

The genus *Piptocephalis* was first established by de Bary in 1865 with the type species *P. freseniana*. It is an obligate parasite of other fungi and with the exception of *P. xenophila* Dobbs and English all the species are known to attack only other members of the Mucorales. Till 1900 twelve species of this genus were described by different workers. Except Naumov (1939) who recognized all the twelve species, different monographers of the genus have recognized varying numbers of species in this genus (Fischer, 1892—8; Lendner, 1908—9; Zycha, 1935—7). The species that have been described since then are *P. macrospora* van Beyma (1944, p. 42) *P. xenophila* Dobbs and English (1954, p. 375), *P. dichotomica* Krzemieniewska and Badura (1954, p. 733), *P. virginiana* Leadbeater and Mercer (1957, p. 461) and *P. lepidula* (Marchal) Benjamin (1959, p. 345). Dobbs and English (1954) have recently discussed the nomenclature of its sporing parts and have suggested some suitable names for them. The several criteria stressed in the keys of van Tieghem (1875), Fischer (1892), Zycha (1935) and Naumov (1939) are (*i*) presence or absence of stolons and rhizoids, (*ii*) presence or absence of sporophore striations, (*iii*) nature of the head-cell, (*iv*) size and shape of the spore and (*v*) number of spores per merosporangium. Leadbeater and Mercer (1956, 1957a, 1957b) and more recently Benjamin (1959) have discussed the value of these different criteria in the taxonomy of this genus and have suggested some additional ones which might prove to be supplementary criteria of taxonomic importance. Leadbeater and Mercer (1957, p. 115) are of the opinion that zygospores are an important supplementary taxonomic criterion and where there are no diagnostic morphological features they may be a primary criterion for speciation. On the other hand, Benjamin (1959) has emphasized on the taxonomic importance of certain characters which had received little attention in the past viz., the presence or absence of the septa in the stipe and branches of the fruiting structures and the nature of the septa, if present.

In India the genus *Piptocephalis* has never been reported. The author came across a species of *Piptocephalis* parasitising *Mucor hiemalis* Wehmer growing on the dung of a wild rat at the Agriculture Farm, Botany Department, University of Allahabad. It was grown in pure mixed culture first on Hay agar and subsequently transfers were made to other media which included potato-dextrose agar (PDA). Abundant zygospores were formed on PDA. Unfortunately after few months of study it was noticed that the parasite was not appearing in fresh transfers. However, material from the old Petri dishes and slides prepared from it have been preserved. The fungus has been found to be not identical with any member of this genus hitherto described, and for which the following name is proposed after the distinguished mycologist Anton de Bary who first described the genus *Piptocephalis*.

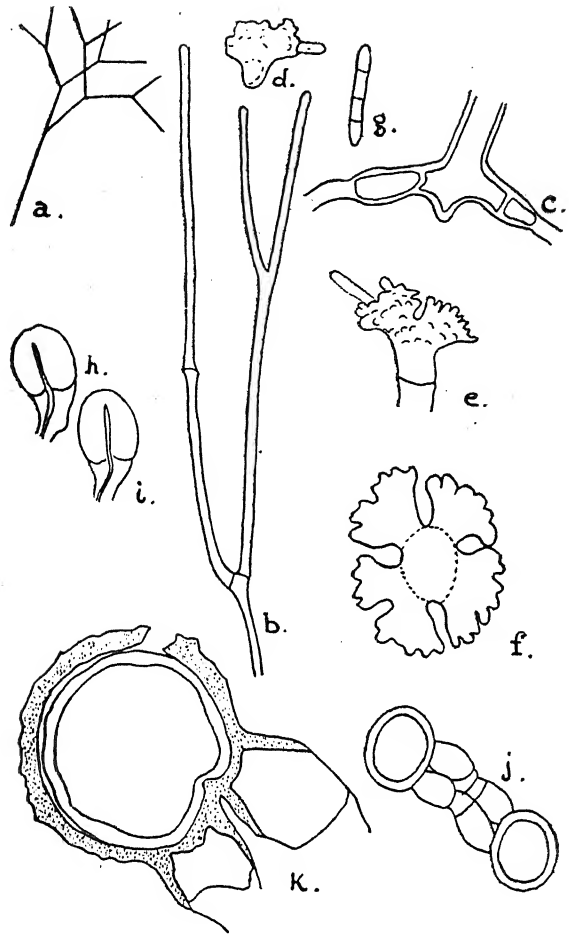


Plate 1. *Piptocephalis de-baryana* sp. nov

Figs. (*a*) Top portion of the sporophore showing the branching pattern. (*b*) A part of the sporophore with an abnormal ultimate branch on the left. ×600. (*c*) A base of a sporophore. ×1500. (*d*) A head-cell with a single spore attached to it. ×1500. (*e*) A magnified side view of a head-cell. ×2400. (*f*) A magnified top view (slightly flattened) of a head-cell. ×2400. (*g*) A merosporangium with four spores. ×1500 (*h, i*) Two figs. showing a stage in zygospore formation. Note the slightly heterogamic character of gametangia. ×600. (*j*) Two mature zygospores originating from the same point. ×600. (*k*) A mature zygospore cracked to show the exospore and endospore. ×1600.

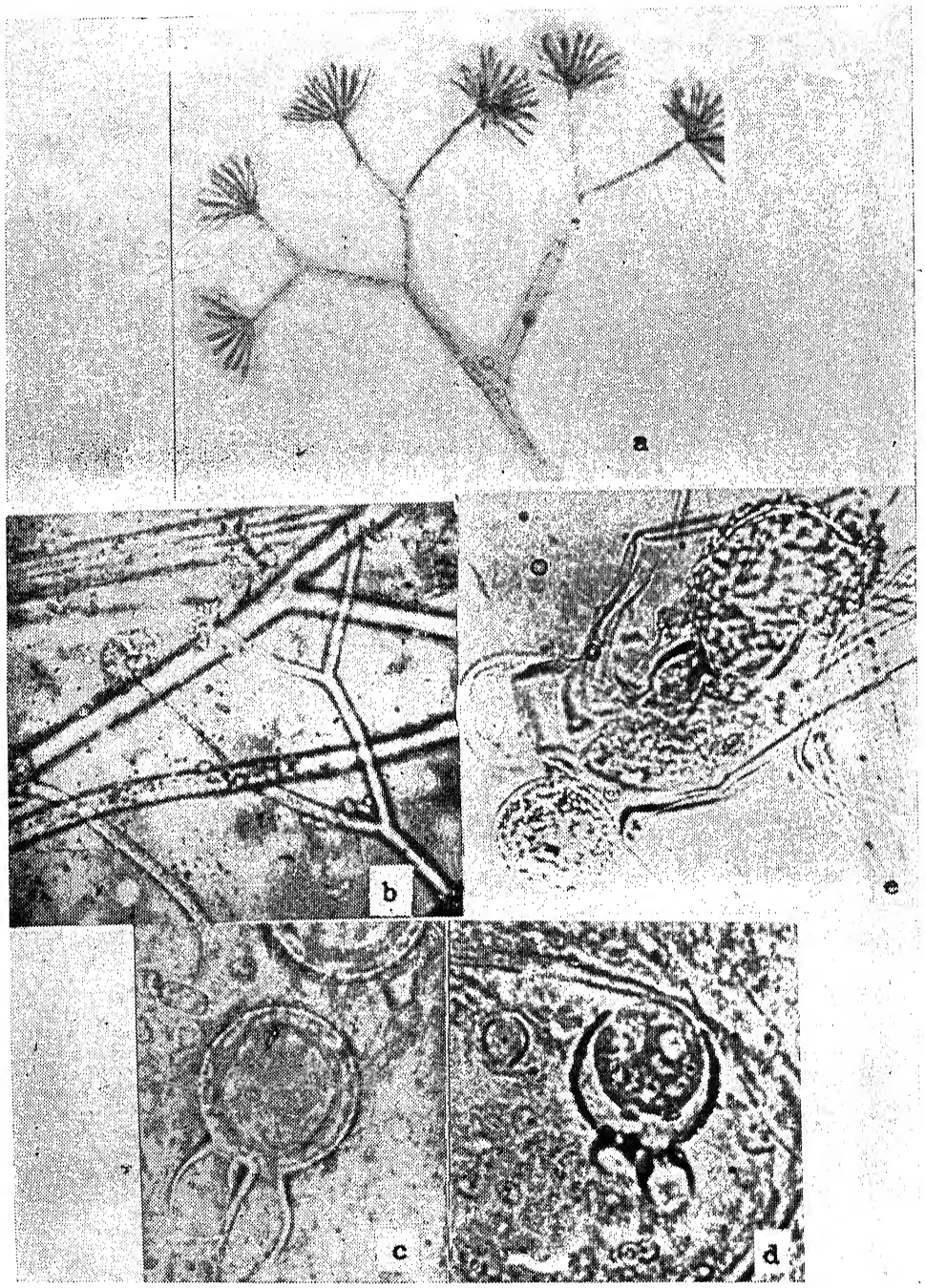


Plate 2. *Piptocephalis de-baryana* sp. nov.

(*a*) Photomicrograph showing terminal portion of a sporophore. Note the branching pattern and the numerous merosporangia attached at the end (head-cells not clearly seen) ×600. (*b*) Photomicrograph showing the ultimate branches with the naked head-cells attached at the end. ×900. (*c*) Photomicrograph of a zygospore ×860. (*d*) Photomicrograph of a mature zygospore carefully crushed to show the smooth endospore emerging from it. ×760. (*e*) Photomicrograph of a completely crushed zygospore showing the rough walled exospore

Piptocephalis de-baryana sp. nov.

Mycelium running over the host hyphae, attached to them at intervals by swollen suckers. Sporophores in mass tan coloured against the background of the host, erect or ascending, 5-15 mm. in length upright with the upper 1/5 th with 5—8 branches at the tip, lower portions of the sporophore approximately 4·5 μ in diameter, higher up 2·5 μ, still higher up in the ultimate branches 1·5 μ but at the tips enlarging to 2·8 – 3 μ. Sporophore mostly nonseptate except at the bifurcations, later becoming longitudinally striate; the fertile branch system variously branched with branches differing or equal in length. The ultimate branches 33-84 μ rarely upto 200 μ in length, not tapering but slightly swollen at apex. Head-cells deciduous, mostly 10 – 10·5 μ wide, obconic with bases broad and plane ; broader at the apex, highly dichotomised, with the margins slightly crenate at the insertion places of the merosporangia, the latter numerous borne on each head-cell, 12-13 μ long with generally 4 cylindrical spores, sporangiospores 2·8 – 5·6 × 1·4 – 1·6 μ (average 4 × 1·4 μ), smooth and colourless. Zygospores golden-yellow or yellow—brown, subspherical to slightly oval, 18 – 34 μ (mostly between 27-30 μ) in diameter, formed as bud-like enlargements from the fused apices of slightly unequal gametangia borne terminally on apposed progametangia ; suspensors smooth, attenuated at the point of attachment with the zygospore ; exospore wall with scattered fine tubercles or echinulations ; endospore wall smooth, about 24 μ thick. Azygospores also frequently seen. (Plate I, Figs. a-k. Plate II, Figs 1-4)

Isolated from the dung of a wild rat at the Agriculture Farm, Botany Department, University of Allahabad, January 15, 1959. Type : Slide mounts and material kept at the Botany Department, University of Allahabad, No. M. 28.

Mycelium decurrens super hyphas hospitis episdemque fixum ad intervalla haustoriis tumescentibus. Sporophori in massa badii, erecti vel ascendentes, 5 – 15 mm. longi, erecti quinta parte superiore 5-8 ramulis ornata ad apicem, partibus inferioribus approx. 4·5 μ diam., superioribus vero 2·5 μ, supremis ad ultimos ramulos 1·5 μ, ad apices vero usque ad 2·8-3 μ. Sporophori vulgo nonseptati praeter bifurcationes, postea evadentes striati ; systema ramulorum fertilium varie ramosum, ramulis longitudine inaequalibus. Ultimi vel celluarum capituli ramuli 33 – 84 μ, raro ad 200 μ longi, non fastigati sed leviter tumescentes ad apicem. Cellulae capituli deciduae, vulgo 10 – 10·5 μ latae, obconicae, basi lata et plana, latiores ad apicem, alte dichotome furcatae, marginibus leviter crenatis ad insertionem merosporangiorum, quae plurima sunt insidentia singulis cellularum capitulis, 12-13 μ longa, vulgo sporis cylindricis quaternis ornata ; sporangiospori 2·8 – 5·6 × 1·4 – 1·6 μ (ut plurimum 4 × 1·4 μ), leves et incolori. Zygosporae aureo-luteae vel luteo-brunneae, subsphaericae vel leviter ovatae, 18-34 μ (vulgo 27-30 μ) diam., efformatae alabastri instar in apicibus fusis gametangiorum leviter inaequalium terminaliter insidentium progametangiis appositis ; suspensores leves, attenuati ad punctum insertionis zygosporarum; exosporium ornatum tuberculis pulchris vel echinulationibus ; endosporium leve, ca. 24 μ crassum. Azygosporae quoque frequentes.

Typus lectus in stercore muris silvatici in praedio Sectionis Botanices Universitatis Allahabadensis mense Januario anni 1959 et servatus in praeparatione microscopica in eadem Sectione eiusdem universitatis sub numero M. 28.

This species has the following peculiar features:

1. The small highly dichotomized head-cells with short, crowded dichotomies.
2. The unusual length of the ultimate (head-cell) branches, ranging from 33-84 μ, rarely upto 200 μ in length. They are often longer than those of any species so far studied.

3. The spores 2·8-5·6 × 1·4-1·6 μ (average 4 × 1·4 μ) are the smallest spores recorded for species having lobed head-cells.
4. The zygospores are smaller, 18-34 μ (mostly 27-30 μ), with sharply attenuated suspensors at the point of attachment to the zygospore.

Since the fungus is no longer alive a more detailed study of the fungus, such as ascertaining its homothallic or heterothallic character, its range of parasitism, could not be undertaken. However, it is proposed to undertake such a study if and when the fungus is reisolated.

ACKNOWLEDGMENTS

The author is deeply indebted to Dr. R. K. Benjamin and Dr. G. Leadbeater for their kind help and useful suggestions. To Dr. C. W. Hesseltine, Dr. R. K. Saksena and Dr. R. N. Tandon the author is grateful for the encouragement they kindly gave in this study. Thanks are also due to the U. P. Scientific Research Committee for the contingency grant and Prof. Fr. H. Santapau for the Latin diagnosis.

REFERENCES

Bary Anton de. 1865. Zur kenntniss der Mucorinen. *Abhandl. Senckenb. Naturf. Ges.* 5 : 345 – 375.

Benjamin, R. K. 1959. The merosporangiferous Mucorales. *Aliso* 4 : 321 – 433.

Beyma Thoe Kingma, F. H. van. 1944. Beschreibung einiger neuer Pilzarten aus dem Centraalbureau voor Schimmelcultures Baarn (Nederland). Antonie van Leeuwenhoek. *Jour. Micro. biol. Serol.* 10 : 41 – 56.

Dobbs, C. G. and Mary P. English. 1954. *Piptocephalis xenophila* sp. nov. parasitic on non-mucorinine hosts. *Trans. Brit. mycol. Soc.* 37 : 375 – 389.

Fischer, A. 1892. Phycomycetes, in Rabenhorst, L., Kryptogamenn – Flora von Deutschland, Oesterreich und der Schweiz. *Zweite Auflage.* 1 (4) : 1 – 505.

Krzemieniewska, H. and L. Badura. 1954. A contribution to the knowledge of the microorganisms from the litter and soil of the beech-wood. *Acta Soc. Bot. Poloniae,* 23 : 727 – 781. (Polish with English summary)

Leadbeater, G. and C. Mercer. 1956. Zygospores in *Piptocephalis cylindrospora* Bain. *Trans. Brit. mycol. Soc.,* 39 : 17 – 20.

——— ——— and ——————— 1957a. Zygospores in *Piptocephalis. Trans. Brit. mycol. Soc* , 40 : 109 – 116.

——————— and ——————— 1957b. *Piptocephalis virginiana* sp. nov. *Tran. Brit. mycol. Soc.,* 40 : 461 – 471.

Lendner, A. 1908. Les Mucorinees de la Suisse in Beitrage zur Kryptogamenflora der Schweiz 3 (1) : 1 – 180. K. – J. Wyss, Libraire-Editeur, Berne.

Naumov, N. A. 1939. Cles des Mucorinees (Mucorales). Encyclopedie Mycologique 9 : 1 – 137 + Appendix. Paul Lechevalier, Paris. Van Tieghem, P. 1875. Nouvelles recherches sur les Mucorinees. *Ann. Sci. Nat., Bot., Ser.* 6 (1) : 5 – 175.

Zycha, H. 1935. Mucorineae, in Kryptogamenflora der Mark Brandenburg 6a : 1 – 264. Gebruder Borntraeger, Leipzig.

COMPOSITION OF SOME FORESTS IN THANA DISTRICT, BOMBAY

By

S. K. JAIN* *and* S. M. KARMARKAR†

Botanical Survey of India, Poona

[Received on 15th June, 1960]

SUMMARY

The composition of some deciduous forests of Thana district has been studied.

The typical deciduous forest is composed of *Tectona grandis—Terminalia tomentosa* community. The common associates are *Anogeissus latifolia* and *Butea monosperma*. *Adina cordifolia* and *Schleichera oleosa* are frequent in Vihigaon area.

The second storey consists mainly of *Erythrina variegata* var. *orientalis*, *Salmalia malabarica*, *Butea monosperma*, *Wrightia tinctoria*, *Acacia chundra*, *Bauhinia racemosa* and *Zizyphus rugosa*. The shrub layer consists chiefly of *Flacourtia* species, *Woodfordia fruticosa*, *Meyna laxiflora*, *Carissa congesta* etc.

The commonest climbers in the area are:— *Calycopteris floribunda*, *Combretum ovalifolium*, *Hemidesmus indicus*, *Mucuna prurita*, *Cryptolepis buchanani*, *Dioscorea bulbifera*, *Dioscorea pentaphylla*, and some cucurbitaceae plants. *Dendrophthoe* species are met with on a number of trees, chiefly on *Terminalia tomentosa*.

The Tectona-Terminalia community appears to be the climatic climax in this area.

INTRODUCTION

The dediduous forests of Bombay State are economically important on account of the commercial value of Teak and Terminalia timber. Studies on the composition of these forests were therefore initiated few years ago. It was also considered desirable to study the ecological status of this community in some selected spots with special reference to the regeneration of the important timber species.

The present study was made at Kasara Ghats in Thana District approximately at 73·20° E. Long. and 19·40° N. latitude, Map. Fig. (1).

---

* Now Economic Botanist, Central Botanical Laboratory, Allahabad.

† Now on staff of Ruia College, Bombay.

The entire area is hilly. Kasara (Washala) is situated almost at the foot of the Thal Ghats. Vihigaon and Thakurwadi forests spread on small hills in the middle of the ghats. Vasind (Photo 1) is an undulating plateaux with small hillocks scattered here and there spreading in the plain country to the west of the ghats. In most cases the hillocks slope to the north-west and south-east sides.

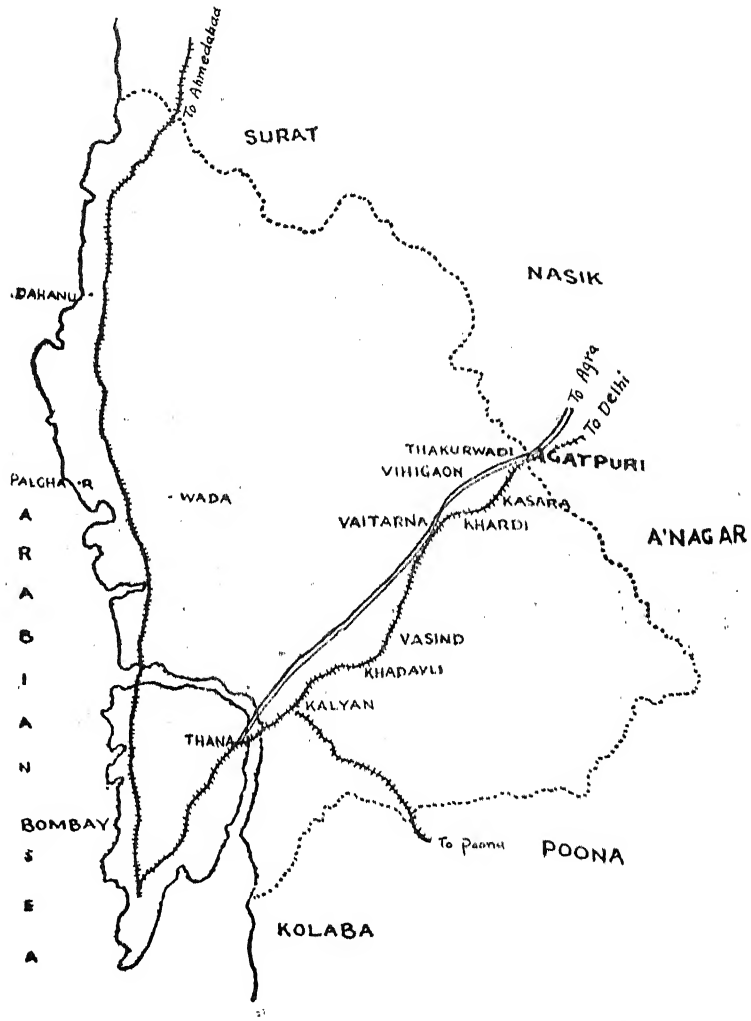


Fig. 1. Map showing Thana District in Bombay State.

The area receives a fairly heavy rainfall from the south-west monsoons. The annual rainfall is approximately 100 inches. As will be seen from the following table, the rainfall occurs chiefly during June-August and except for these monsoon months, the remaining period is dry. Data about rainfall are available only for Kalyan and Igatpuri. Kalyan is in south-west and Igatpuri in north-east of these forests.

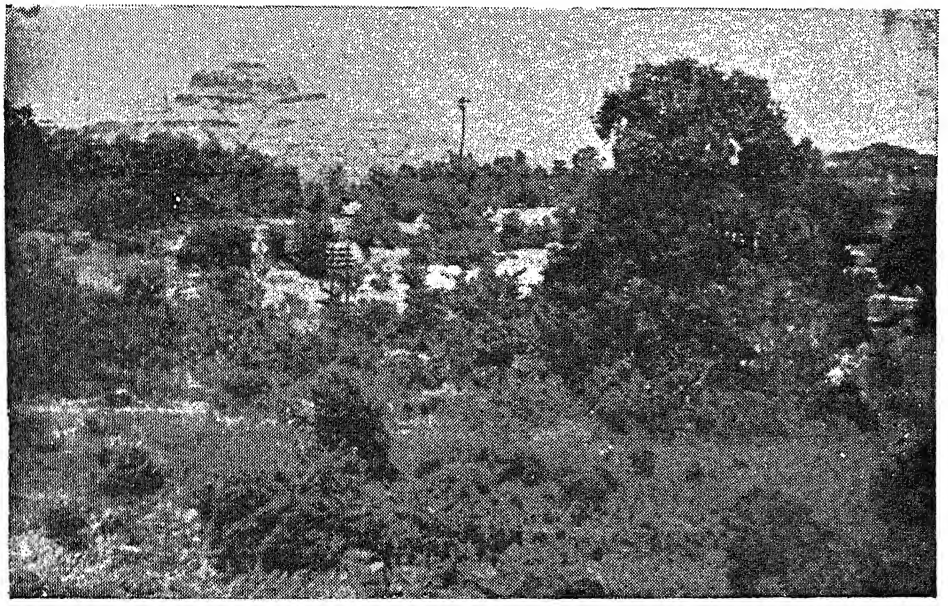


Photo 1. A view of Forest and Hills, Vasind.

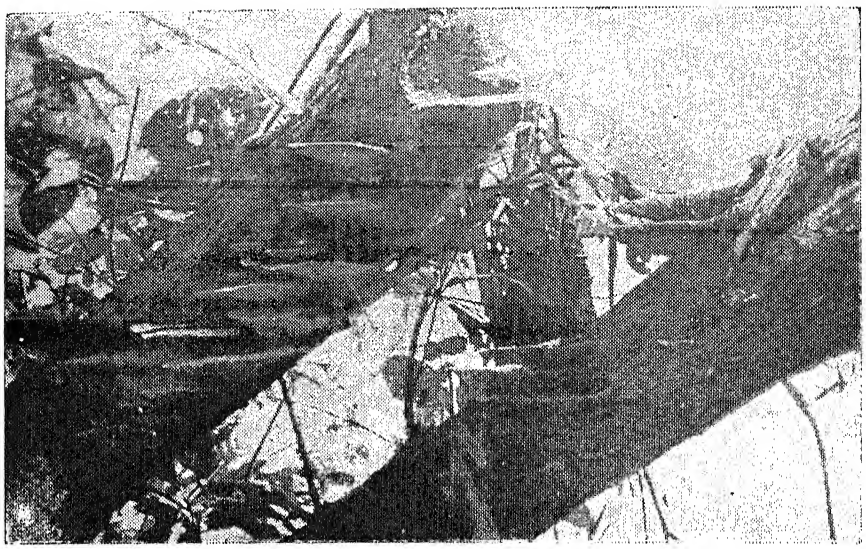


Photo 2. Fungi on dead wood of *Terminalia*, at Vasind.

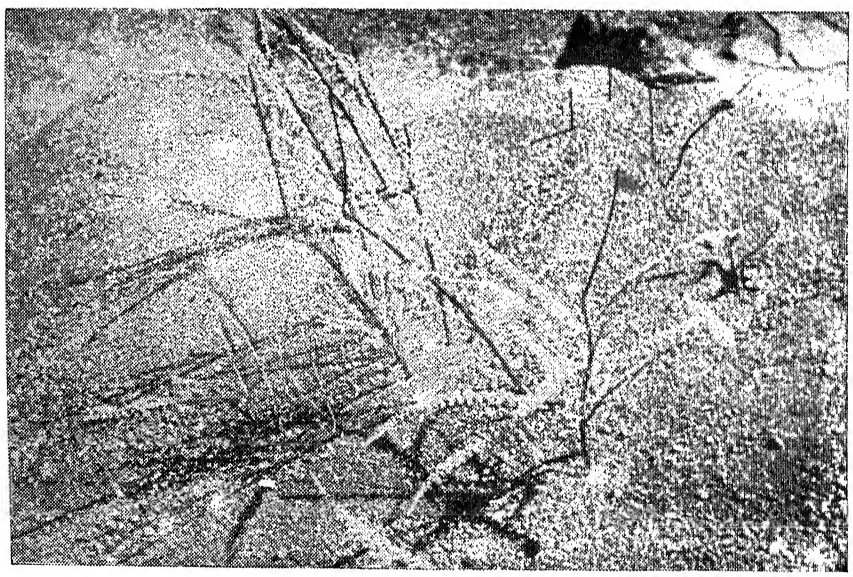


Photo 3. Plants of *Tamarix ericoides* in the sandy bed of river near Vasind.

MONTHLY RAINFALL IN INCHES

	Kalyan	Igatpuri
January	·17	·1
February	·8	·05
March	·03	·02
April	·02	·19
May	·47	·84
June	18·8	20·21
July	37·04	51·09
August	23·08	36·53
September	12·06	17·17
October	3·43	4·47
November	·42	·72
December	·1	·14
Annual	95·7	131·53

BIOTIC INFLUENCES

These forests are under the Forest management and are mostly reserved. The hill tops are used for habitation and farming and thus interference on hill tops is evident. Grazing, browsing and illicit lopping are common and in some cases large patches have been cleared and are now lying barren. Especially at Vaitarna, in the catchment area, forests have been cleared to accommodate the Vaitarna Camp.

VEGETATION

The vegetation was studied by running a number of transects across the main topographical features, hills and valleys, at a number of places. Actual recordings were done in quadrats varying from 6-8 meter diam. according to the density of the vegetation. Ground flora species were recorded in smaller quadrats. Plants growing in this area in different seasons were collected and identified at the Herbarium of the Botanical Survey of India, Western Circle, Poona.

A summary of the data, showing the composition of different plant populations is given in table 1.

TABLE 1

Showing percentage occurrence of different species in quadrats

Area	Kasara	Vasind Khadavli area	Kasara Washala Range	Vihigaon, 3½ miles from Thalghat	Thalghat, near Rest House	Thakurwadi top to 01 Nala	Dahigaon on way to Vaitarna
Rock and Geology	Trap	Trap	Trap	Trap rock	Trap rock	Trap rock	Trap rock
Soil	Coarse red c ayey shallow soil	Shallow dry pebbly reddish brown	Coarse red clayey soil	Red clayey soil	Reddish clayey soil	Gravelly coarses oil	Coarse gravelly soil
Aspect	East & South-east	Almost plain country	East & South-east	North-eastern slope	South western slope	Southern	Western
Biota	Reserved forest, but subject to grazing	Open to grazing; lop-ping also seen	Reserved forest, subject to grazing	Reserved forest, but lopping & grazing evident	Reserved forest; road passes through this area	Reserved forest, subject to grazing	Reserved forest, subject to grazing
Particulars of Quadrats	6 meter diam.	6 meter diam	6 meter diam.	8 meter diam.	6 meter diam.	6 meter diam.	6 meter diam.
1	2	3	4	5	6	7	8
Trees	%	%	%	%	%	%	%
Adina cordifolia Hk.	...	...	10	40	...	...	5
Anogeissus latifolia Wall.	...	26	30	30	38	42	15
Bauhinia racemosa Lamk.	...	.	...	...	13	18	22
Boswellia serrata Roxb.	...	7	...	40	...	...	...
Bridellia sp.	...	20	30	...	...	6	13
Butea monosperma O.K.	82	24	20	...	...	6	25
Casearia tomentosa Roxb.	...	...	...	...	13	...	...
Cassia fistula L.	...	7	...	...	...	...	...

Dalbergia Sp.	...	...	...	...	...	...	5
Dillenia pentagyna R.	17	...	30	10	...	...	...
Erythrina variegata var. orientalis Merr.	17	20	10	40	...	...	...
Ficus sp.	...	...	...	...	...	6	...
Flacourtia sp.	...	...	20	10	25	24	19
Grewia tiliaefolia Vahl	...	34	...	...	---	...	...
Gymnosporia sp.	...	13	...	---	...	...	...
Hymenodictyon excelsum Wall.	17	...	...	...	...	...	---
Lannea coromandelica Merr.	34	7	...	---	...	...	...
Madhuca indica Gmel.	...	...	30	...	...	...	15
Mitragyna parvifolia Korth.	...	...	10	10	...	12	...
Oroxylum indicum Vent.	...	...	...	...	25	...	...
Pouteria tomentosa (Roxb.) Baehni	34	...	...	...	---	...	...
Radermachera xylocarpa Schum.	...	---	---	...	13	...	...
Randia dumetorum Hk.	17	...	...	...	...	...	...
Salmalia malabarica Sch. & Endl.	17	13	10	...	...	...	5
Schleichera oleosa Oken.	...	...	...	20	...	6	5
Syzygium cuminii Skeels	...	...	10	...	...	...	...
Tectona grandis L.f.	100	51	70	90	63	...	56
Terminalia arjuna W. & A.	50	...	---	...		...	...
Terminalia bellerica Roxb.	...	...	...	...	...	6	8
Terminalia tomentosa Clke.	67	57	70	30	38	30	95
Wendlandia exserta DC.	...	...	10	...	...	...	...
Wrightia tinctoria R. Br.	...	...	10	...	25	12	15
Shrubs							
Acacia chundra Willd.	...	7	...	...	...	...	20
Ampelocissus latifolia Planch.	50	...	...	...	...	...	...
Calycopteris floribunda Lamk.	...	...	...	20	...	...	...
Capparis Sp.	---	...	...	...	50	...	---
Combretum ovalifolium R.	...	13	...	10	---	...	---
Dendrophthoe Sp.	34	...	10	...	...	...	...
Derris Sp.	...	7	...	...	---	...	...
Emblica officinalis Gaertn.	---	...	20	...	...	...	10
Jasminum sp.	...	...	...	...	...	18	...
Leea crispa L.	17	13	...	...	...	...	...
Meyna laxiflora Robyns.	34	...	40	40	75	30	13
Pavetta indica L.	...	...	...	...		12	...
Pogostemon parviflorus Bth.	...	...	...	...	...	...	5
Vitex negundo L.	...	...	...	...	...	6	...
Woodfordia fruticosa Kurz.	...	...	30	...	...	5	...

The forest consists of Teak-Terminalia community which is almost uniform in composition over most of the area. *Anogeissus latifolia* and *Butea monosperma* occur as associates in most of the areas. Trees are generally tall. Common tree species are *Terminalia tomentosa, Terminalia bellerica, Terminalia arjuna, Mitragyna parvifolia,* and *Bridelia.* The various species of *Terminalia* are economically important both as timber and also for their medicinal properties. *Lagerstroemina* spp, *Salmalia malabarica, Madhuca indica, Randia dumetorum* and *Gymnosporia* sp. are restricted in their distribution. *Adina cordifolia* and *Schleichera oleosa* are very common at Vihigaon forests. *Dillenia pentagyna* occurs in some places. In some spots particularly moist situations, the presence of *Mangifera indica, Syzygium cuminii* and *Pongamia pinnata* is conspicuous. At Kasara *Wrightia tinctoria, Cassia fistula, Dillenia pentagyna, Boswellia serrata* and *Salmalia malabarica* are also met with.

Meyna laxiflora and *Carissa congesta* are the dominant shrubs and are responsible for giving a green colour to an otherwise dry forest in the summer months. At Vasind *Carissa* occurs on the fringes of the forest whereas at Kasara its distribution is restricted to the middle of the forest and on rocky walls. Other common shrubs here are species of *Ampelocissus, Cissus, Leea, Zizyphus,* and *Woodfordia. Ampelocissus latifolia* and *Leea crispa* occur at Kasara. The commonest species of *Zizyphus* are *Zizyphus rugosa* and *Zizyphus xylopyrus.*

The trees of *Schleichera oleosa* with their copper coloured young leaves give a characteristic appearance to the forest when most of it is barren. *Woodfordia fruticosa* is common on rocks or on the sides of dried nalas.

Species of *Dendrophthoe* are frequently seen occuring as parasites on a number of trees, mainly *Terminalia tomentosa.*

Bamboos are abundant along river bank at Vaitarna.

As the forests are not very dense, the climbers are few viz. *Hemidesmus indicus, Cryptolepis buchanani, Dioscorea* spp. etc. At Vihigaon, *Combretum ovalifolium* and *Calycopteris floribunda* are common climbers. *Mucuna prurita* is common at Vasind.

Seedlings of a number of plants such as *Adina cordifolia, Butea monosperma, Terminalia tomentosa, Cassia fistula, Bauhinia racemosa, Erythrina variegata* var. *orientalis, Carissa congesta* and *Tectona grandis* are frequently present in those forests.

Numerous other erect or climbing shurbs occur but they do not have a wide distribution e.g. *Acacia chundra, Capparis* sp., *Combretum* sp., *Calycopteris floribunda, Derris* sp., *Pavetta indica, Emblica officinalis* and *Vitex negundo.*

The ground vegetation shows a distinct seasonal aspect. The monsoon flora is very rich while a very few herbs, chiefly grasses, grow in the dry season.

The monsoon flora affords a rich variety of plants, *Alysicarpus* and *Justicia simplex* along with *Cassia tora* are commonly seen. Another species of *Cassia—C. absus* has only restricted distribution. Other common herbaceous plants are species of *Phaseolus, Justicia, Sida, Commelina, Rungia, Impatiens, Crotalaria, Daedalacanthus* and *Anotis lancifolia, Euphorbia, hypericifolia, Euphorbia hirta, Hypoxis aurea, Hibiscus* sp., *Indigofera tinctoria, Tephrosia purpurea, Phyllanthus niruri, Urena lobata,* etc. etc.

At the beginning of the monsoon season *Crinum* species and *Curcuma pseudomontana* are common. In some areas *Crinum* species are dominant in a

Crinum—Cassia tora community. *Hypoxis aurea* is also very common. *Curcuma pseudomontana* occurs on slopes and flowers in the early monsoon period.

The ground Orchid *Habenaria grandiflora* occurs on slopes in large numbers.

At Vihigaon *Vitex negundo, Jatropha* and *Jasminum* sp. are common near habitations.

A number of Bryophytes, Fungi and Pteridophytes also occur during monsoon. *Anthoceros, Adiantum* and *Ophioglossum* were collected at Kasara. Bracket fungi and Mushrooms are also common (Photo 2).

These forests are very poor in ground flora during other periods. In the summer months only stumps of dried grasses are left by cattle. Common grasses are species of *Arundinella, Apluda, Capillipedium, Ischaemum, Heteropogon* and *Themeda.* Other plants include species of *Cocculus, Malachra, Urena, Daedalacanthus, Haplanthus* and some sedges.

Around Kasara, vegetation near and in ponds consists mainly of *Caesulia axillaris, Alternanthera sessilis, Ludwigia parviflora, Jussiaea* and *Polygonum. Tamarix ericoides* occurs in sandy dried river beds (Photo 3).

The study of the forests of this area is being continued further. The present Teak-Terminalia community appears to be the climatic climax here. The flora does not show much variety at the different spots studied so far.

ACKNOWLEDGEMENT

We are very grateful to Dr. J. C. Sen Gupta and Dr. G. S. Puri for kindly granting us all facilities and help for this work.

BIBLIOGRAPHY

Cooke, T. 1908. The Flora of Bombay, Reprinted 1958, I—III, Calcutta.

Hooker, J. D. 1872—97. The Flora of British India, I—VII, London.

Jain, S. K. 1960. Distribution patterns of some plants of Deccan Trap. *Mem. Indian bot. Soc.* (in Press).

Jain, S. K. and Karmarkar, S. M. 1958. The Dry Deciduous forests of Thana, *Proc. Indian Sci. Cong.* Pt. III, 324.

Misra, R. and Puri, G. S. 1957. Indian Manual of Plant Ecology, Dehra Dun.

Puri G. S. & Jain, S. K. 1958. Scrub vegetation under Forest Management at Dhond, Bombay. *Proc. Nat. Inst. Sci.* Vol. 24, B. (3) 145—149.

Puri, G. S. and Jain, S. K. 1959. Flora of the Deccan Trap. *Proc. Indian Sci. Cong.* Pt. III, 293.

SOME PHYSIOLOGICAL STUDIES ON TWO SPECIES OF *CUNNINGHAMELLA*

By

R. K. SAKSENA *and* A. K. SARBHOY

Botany Department, University of Allahabad, Allahabad

[Received on 17th June, 1960.]

INTRODUCTION

All the micro-organisms including fungi have a definite pattern for growth and their development depends on the environmental and nutritional conditions. Amongst the former conditions temperature is one of the important factors controlling growth and sporulation of micro-organisms. It has been found that for different organisms different minimum, optimum and maximum temperatures for growth and sporulation are necessary. Out of the latter conditions nitrogen and sulphur are important constituents of a suitable nutrient medium. Considerable amount of work has been done in the past dealing with nitrogen and sulphur requirements of fungi including some *Mucorales* and different results have been obtained by different workers. Hagem (1910) reported that ammonium nitrate, ammonium chloride and sodium nitrite were good sources for many members of *Mucoraceae* and Raizada (1952, 1957) also got similar results in case of *Mucor hiemalis*, *Mucor fragilis* and *Cunninghamella echinulata*, whereas these were found moderate or poor sources of nitrogen for *Absidia cylindrospora* and *Rhizopus sontii*.

The present work was undertaken to study the role of temperature, various nitrogen and sulphur compounds on the growth and sporulation of the two species of *Cunninghamella* viz., *Cunninghamella echinulata* Thaxter and *Cunninghamella bertholletiae* Stadel.

MATERIAL AND METHODS

Cunninghamella echinulata Thaxter and *Cunninghamella bertholletiae* Stadel were isolated by the authors from a soil sample obtained from the Agriculture Farm, Allahabad University.

The studies on temperature experiments were conducted on solid synthetic Mucor Agar ('SMA'; Hesseltine 1954) medium of the following composition : Dextrose 40 gm., Asparagine 2 gm., Potassium acid phosphate 0·5 gm., $MgSO_4$. $7H_2O$ 0·25 gm., Thiamine Chloride 0·5 m. gm., Distilled water 1,000 c.c. Bacto - agar 2%.

Ten c.c. of the medium were poured in Petri dishes (10 c.m. in diameter). In order to remove the lag effect the dishes were kept at the particular temperature for a fixed period before inoculation. The temperature ranged from 10°C to

45°C with an interval of every 5°C. The margins of actively growing colony on a solid medium were uniformely cut off into discs of 4 m. m. in diameter and each dish was inoculated with one of these. The dishes were then incubated for 48 hours and measurements of radial growth were taken after every 24 hours. All the experiments were run in triplicates and dishes showing any contamination were discarded.

For experiments on nitrogen requirements the liquid 'SMA' medium of the above composition without the nitrogen source (*i.e.* minus asparagine) acted as the basal medium. Various nitrogen compounds (inorganic and organic) listed below were added singly to the basal medium in amounts calculated to furnish 372 m. gms. of nitrogen per litre. The pH of the medium was adjusted to 6·8 before autoclaving.

Inorganic: Sodium nitrite, Potassium nitrate, Ammonium nitrate and Ammonium chloride.

Organic: Glycine, d-alanine, d-valine, Glutamic acid, Asparagine and Acetamide.

For studies on sulphur requirements the 'SMA' medium without the sulphur source (*i.e.* minus magnesium sulphate) acted as the basal medium. Various sulphur compounds (inorganic and organic) listed below were added singly to the basal medium so as to furnish 32 m. gms. of sulphur per litre. The amount of sulphur present in very small quantities of thiamine chloride added was neglected. Cystin was tried in 1% concentration because of its low solubility.

Inorganic: Potassium sulphate, sodium sulphite, sodium thio-sulphate and sodium bi-sulphite.

Organic: Cystin, Methionine and Thio-urea.

The different liquid media were poured in 25 c.c. quantities in 150 c.c. Erlenmeyer Pyrex flasks and autoclaved at 15 lb. pressure for 15 minutes. The Pyrex flasks, which were used in the experiments, were thoroughly washed and cleaned with hot distilled water. After an interval of every three days one of the flasks was taken from each set of different nitrogen and sulphur compounds for study. The pH of the media was ajusted to 6·8 before autoclaving.

When the incubation periods were over, the contents of the flasks were filtered, fungal mats were washed, dried and weighed for the determination of dry weights. The filterate in each case was used for the determination of change in pH of the medium. For this purpose B. D. H. standard pH papers were used. The dry weights of the fungal mats were statistically analysed.

RESULTS AND DISCUSSION

The comparative data of the diametric spread in cms. of the fungi incubated at different temperatures are given in Table 1.

TABLE 1

Showing the diametric spread (in cms.) of the fungal colonies of *Cunninghamella echinulata* and *bertholletiae*.

Temperature	Diametric spread of					
	Cunninghamella echinulata			*Cunninghamella bertholletiae*		
	24 hrs. in cms.	48 hrs. in cms.	Daily advance in cms.	24 hrs. in cms.	48 hrs. in cms.	Daily advance in cms.
10°C	·3	·5	·8	·3	·5	·8
15°C	·4	·8	1·2	·5	·9	1·4
20°C	·6	1·2	1·8	·8	1·0	1·8
25°C	·8	1·4	2·2	·9	1·5	2·4
30°C	·8	1·6	2·4	1·0	1·8	2·8
35°C	1·9	2·6	4·5	1·2	2·1	3·3
40°C	·8	2·2	3·0	·7	1·2	1·9
45°C	·7	1·1	1·8	·5	1·0	1·5

It is evident from Table 1 that both the species grew poorly at 10°C (the lowest temperature used in the present series). Growth increased with the increase in temperature upto 35°C, beyond which the growth started to decline. Very little growth was observed at 45°C. The optimum temperature for the growth of both the species was 35°C. In case of *Cunninghamella bertholletiae* there was a sharp decline in the growth rate at temperatures higher than 35°C in contrast to that of *Cunninghamella echinulata*.

The two species of *Cunninghamella* have a minimum temperature for the growth below 10°C. Tisade (1917) with *Fusarium lini* and Fawcett (1921) with *Phytophthora terresteris* reported that minimum temperatures for the growth of these fungi were 10°C and 12°C respectively. Ray Chaudhri (1942) found that *Diplodia cajani* could not grow at 15°C or below it, similarly the lower limit of temperature range for *Diplodia natalensis* (Ramsay *et al* 1946) was 17·7°C (50°F).

The results on nitrogen requirements are given in Table 2.

TABLE 2

Showing the average dry weight and sporulation of *C. echinulata*, *C. bertholletiae*, on different nitrogen sources and changes in pH values after the growth of the fungus

Room Temperature 19°C to 22°C

Nitrogen compounds	*C. echinulata*			*C. bertholletiae*		
	Dry wt. in mgms	Sporulation	pH	Dry wt. in mgms	Sporulation	pH
1. Ammonium nitrate	110·3	good	4·9	119·0	good	5·0
2. Ammonium chloride	84·3	good	5·3	65·0	moderate	5·2
3. Potassium nitrate ...	65·3	moderate	3·4	76·0	moderate	4·5
4. Sodium nitrite ...	85·3	good	6·4	93·0	good	6·3
5. Glycine ...	62·0	poor	4·7	84·6	good	5·0
6. Alanine ...	137·6	good	5·3	83·0	good	5·2
7. Valine ...	81·6	good	5·3	99·6	good	4·2
8. Glutamic acid ...	87·3	good	3·5	60·0	poor	5·3
9. Asparagine ...	83·3	good	4·6	77·6	moderate	5·2
10. Acetamide ...	68·0	moderate	3·7	40·6	poor	4·0
11. Control (no nitrogen)	Nil	Nil	6·2	Nil	Nil	5·8
Average Mean ...	69·58			71·6		

The summary of dry wt. results on the basis of *separate* analysis of these two species is given below :

C. echinulata

Summary of the dry weight results and conclusions at 1% level at P.

Treatments ... Highly significant
Replicates ... Non-significant
S. E. ... 1·48
C. D. at 1% level ± 5·894

Nitrogen Compounds
Dry weight in m. gms

Alanine 137·6 > Ammonium nitrate 110·3 > Glutamic acid 87·3

Sodium nitrite 85·3 — Ammonium chloride 84·3 — Asparagine 83·3 — Valine 81·6 >

Acetamide 68·0 — Potassium nitrate 65·3 — Glycine 62·0 > Control 0·0

C. bertholletiae

Summary of the dry weight results and conclusions at 1% level at P.

Treatments	...	Highly significant
Replicates	...	Non-significant
S. E.	...	0·67
C. D. at 1% level	±	6·668

Nitrogen Sources : Dry weight in m. gms

Ammonium nitrate 119·0 > Valine 99·6, Sodium nitrite 93·0

Glycine 84·6, Alanine 83·0, Asparagine 77·6, Potassium nitrate 76·0 >

Ammonium chloride 65·0, Glutamic acid 60·0 > Acetamide 40·6 > Control 0·0

There was not much difference in the weight of the colonies in replicate cultures. The average values have been tabulated.

The results on sulphur requirements are given in Table 3.

TABLE 3

Showing the average dry weight and sporulation of *C. echinulata*, *C. bertholletiae*, on different sulphur sources and changes in pH values after the growth of the fungus

Room Temperature = 19°C to 22°C

Sulphur compounds	*C. echinulata*			*C. bertholletiae*		
	Dry wt. in mgms	Sporulation	pH	Dry wt. in mgms	Sporulation	pH
1. Potassium sulphate	32·3	good	5·5	31·0	good	3·5
2. Sodium sulphite ...	28·6	good	5·6	26·0	good	5·6
3. Sodium thio-sulphate	25·0	moderate	5·8	24·0	moderate	5·8
4. Sodium bi-sulphite	32·3	good	5·5	27·0	good	5·5
5. Sodium hypo-sulphite	28·0	good	5·4	27·6	good	5·4
6. Cystin ...	21·0	moderate	6·2	18·0	moderate	6·2
7. Methionine ...	18·0	moderate	5·3	19·3	moderate	5·3
8. Thio-urea ...	15·0	poor	5·5	17·3	moderate	5·5
9. Control (no sulphur)	Nil	Nil	6·4	Nil	Nil	6·4
Average Mean ...	22·37			21·24		

The summary of dry wt. results on the basis of *separate* analysis of these two species is given below :

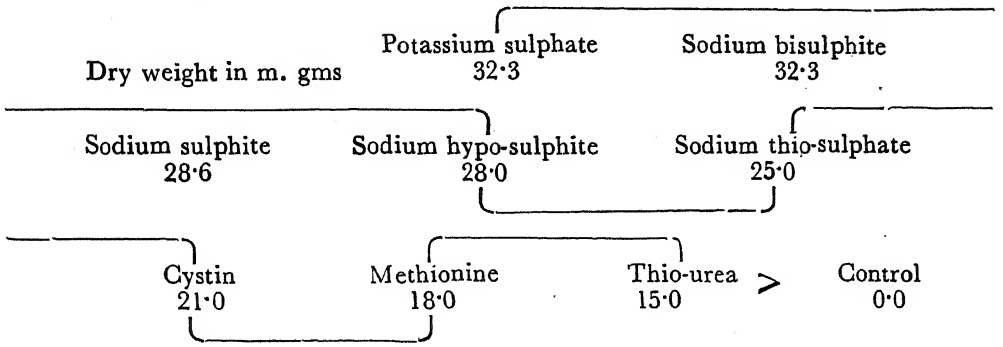
C. echinulata

Summary of the dry weight results and conclusions 1% level at P.

Treatments	...	Highly significant
Replicates	...	Non-significant
S. E.	...	1·14

C. D. at 1% level ± 4·66

Sulphur Compounds :



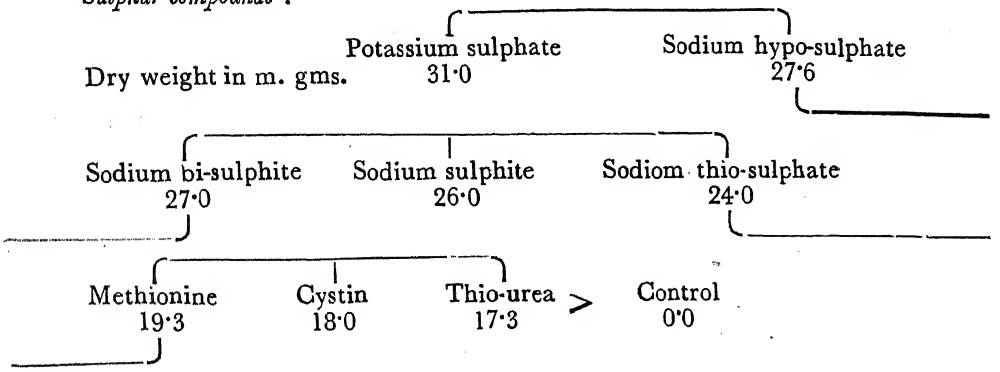
C. bertholletiae

Summary of the dry weight results and conclusions at 1% level at P.

Treatment	...	Highly significant
Replicates	...	Non-significant
S. E.	...	1·128

C. D at 1% level ± 4·61

Sulphur compounds :



In case of nitrogen compounds it was observed that there was no growth in the control lacking in nitrogen. This is in general agreement with the results of

other workers according to which nitrogen is essential for the growth of fungi. Ammonium nitrate was a good source for the growth of *C. echinulata* and *C. bertholletiae*. Similar result was obtained by Saksena (1940) for some species of *Pythium* and by Raizada (1957) for some *Mucorales*, viz. *Absidia cylindrospora* and *Cunninghamella elegans*.

Ammonium chloride supported good growth of *C. echinulata* while it was mediocre for *C. bertholletiae*. In the present studies *C. echinulata* resembled in its behaviour with fungi used by Bhargava (1945) and Mehrotra (1949) who reported its favourable utilization. In case of *C. bertholetiae* it was a mediocre source as reported by Subramaniam and Srinivaspai (1953) for *Fusarium vasinfectum*.

Sodium nitrite supported better growth than potassium nitrate. Nitrite nitrogen has been found to be a suitable source of nitrogen by some workers *e.g.*, Hagem (1910) for many members of *Mucoraceae*. Leonian and Lilly (1939) for *Blakeslea trispora* and Raizada (1957) for *C. echinulata, Mucor hiemalis* and *Mucor fragilis*. Raizada (l. c.) found that nitrites were moderate or poor sources of nitrogen for *Absidia cylindrospora* and *Rhizopus sontii*.

Lemoigne *et al* (1936) reported that the culture solution on which *Aspergillus niger* was grown was capable of reducing nitrites to hydroxylamine. These investigators also studied the ability of this fungus to employ hydroxylamine as a source of nitrogen and they concluded that this compound served as the primary source of inorganic nitrogen for protein synthesis. It may be mentioned here that the present authors also obtained good growth of the above two organisms when they were grown on the basal medium to which hydroxylamine ·35 mgm was added. It thus indicates that the good growth on nitrite nitrogen may be connected with the ability of these organisms to reduce it to hydroxylamine which is then utilized for the synthesis of protein. The present results are similar to those of Lemoigne *et al*.

The two species taken by the authors for the present study appeared to make significantly better growth on ammonium nitrogen than on nitrate nitrogen (potassium nitrate). Similar results were reported by Lopatecki and Newton (1956) for *Phythopthora cactorum* and *Phpthophthora parasitica* and also Raizada for *Mucorales* (1957). Ammonium salts were utilized preferentially by the present organisms. Yemm (1954) reported that in *Torulopsis utilis* ammonium assimilation results in formation of glutamic acid. Other amino acids can be formed from glutamate by transamination. These results of the above workers clearly establish that the ammonium salts are ultimately converted into a mixture of complex organic compounds. In the present case also the good growth on ammonium salts appears to be due to the reasons given by the above workers.

All the three mono-amino-carboxylic acids taken by the authors *viz.*, alanine, valine and glycine proved to be good sources for *C. bertholletiae* but for *C. echinulata* only the first two amino-acids supported good growth while glycine was found to be a poor source. Glycine has been reported to be a good source of nitrogen for *Phycomyces blakesleeanus* (Leonian and Lilly, 1940) and a poor source for several imperfect fungi. e.g., *Fusarium oxysporum, Helminthosporium gramineum* (Gottleib 1946). In general, alanine and valine have been found to be good sources of nitrogen for a number of *Mucorales viz., Choanephora cucurbitarum* (Lilly and Barnett, (1956), *M. rouxii, Absidia cylindrospora, Rhizopus sontii* (Raizada, 1957).

Glutamic acid was found by the authors to be a good source of organic nitrogen for *Cunninghamella echinulata*. Similar results were obtained by Raizada (1957) for the fungi investigated by him. This acid was a poor source for *C. bertholle-*

tiae. In this respect the two species showed marked differences, Kumar (1958) also found it to be a poor source for *Botryodiplodia theobromae* and *Diplodia cajani.*

Asparagine proved to be a good source of nitrogen for *C. echinulata* while it was moderate for *C. bertholletiae.* Leonian and Lilly (1940), Bhargava (1945), Gordon, Patel *et al* (1950). Bilgrami (1956) and Raizada (1957) reported that it was a good source of nitrogen for the organisms investigated by them. It was, however, found to be a moderate source for *C. bertholletiae.* Raizada (1957) had obtained similar results for *Mucor fragilis, Absidia cylindrospora* and *Mucor hiemalis.*

Acetamide supported moderate growth of *C. echinulata* while it was a poor source for *C. bertholletiae.* Acetamide has been reported to be a good source of nitrogen for *Phyllosticta artocarpina* and *Phyllosticta cycadina* by Bilgrami (1956), moderate for *Choanephora cucurbitarum, Rhizopus sontii* and *Mucor rouxii* by Raizada (1957), who found it to be a poor source for *Cunninghamella echinulata, Cunninghamella elegans* and *Mucor fragilis* (1952, 1957).

The results obtained by the authors are in general agreement with those of Foster (1949, page 493) who reported that "Virtually all fungi grew faster and probably more abundantly with complex organic materials as the source of nitrogen than with inorganic nitrogen."

In each case the pH of the medium drifted towards the acidic side with the growth of the fungus; as reported by Raizada (1952, 1957) for the fungi investigated by him.

In the absence of any definite method for ascertaining the degree of sporulation in two species, only the visual observations served as a basis for noting the spore production. Timnick *et al* (1951) also used the same method for finding the degree of sporulation in *Melanconium fuligineum.*

As far as sporulation was concerned, ammonium nitrate was found to be better than sodium nitrite, the sporulation being good in each case. In potassium nitrate there was moderate sporulation of both the species. In ammonium chloride it was moderate in *C. bertholletiae* while good in *C. echinulata.*

Among the three mono-amino-mono carboxylic acids alanine and valine only supported good sporulation while glycine was found to be a poor source for *C. echinulata.* These results are in agreement with those of Lilly and Barnett (1951) for *Choanephora cucurbitarum* as far as alanine and valine are concerned. In case of glycine Lilly and Barnett got good response. The results of the authors, therefore, differ from those of Lilly and Barnett (l.c.). It may be noted that glycine did not support sporulation in case of *C. cucurbitarum* as reported by Raizada (1957).

Glutamic acid and Asparagine were good sources for the sporulation of *C. echinulata,* but the former was poor and the latter a moderate mediocre source for *C. bertholletiae.* These results are supported by Bilgrami (1956) and Raizada (1957) on different fungi used by them.

Acetamide supported poorer sporulation in *C. bertholletiae* than in *C. echinulata,* for which it was mediocre. These results are in agreement with the findings of Bilgrami (1956) and Raizada (1957) for some fungi investigated by them.

In view of the results reported above, the authors support the opinion of Hawker (1950, p. 149) that "No generalization can be made regarding the fruiting in relation to nitrogen sources."

In case of sulphur compounds it was observed that there was no growth in the control lacking in sulphur. This is in general agreement with the results of other workers that sulphur is essential for the growth of fungi. Potassium sulphate, sodium bi-sulphite, sodium sulphite, and sodium-hypo-sulphite were good sources for *Cunninghamella echinulata* and *Cunninghamella bertholletiae*. Best growth on these compounds was observed by many workers *viz.*, Armstrong (1921) for *Aspergillus niger*, *Penicillium glaucum* and *Botrylis cineria*, Bhargava (1945) for *Brivilegnia gracilis*, Tandon (1950) for *Curvularia penniseti*, and Kumar (1958) for *Diplodia cajani* and *Macrophomina phaseoli*. Sodium-thio-sulphate supported only moderate growth as was the case with *Macrophomina phaseoli* (Kumar, 1958).

A review of the existing literature shows that most of the fungi utilize sulphate, sulphite, bi-sulphite, thio-urea and their oxidised forms of inorganic sulphur (Bhargava 1945 and Saksena *et al* 1953). The details of sulphate reduction are not fully known. Davis (1955) however, suggested the following pathway of sulphate reduction sulphate (SO_4^{--}) → Sulphite (SO_3^{--}) → Sulphide (SO_2^{--}) or thio-sulphate ($S_2O_3^{--}$) → Cystin → methionine. The present results show that the three organic sulphur compounds used in the present investigation *viz.*, Cystin and methionine were moderate sources for the growth of both the organism. These results are in agreement with those of Bhargava (1945) and Mehrotra (1949). Thio urea proved to be moderate for *C. bertholletiae* while it was poor for *C. echinulata* indicating that the fungi did not make use of sulphur attached to the carbon.

In both cases sporulation was good in potassium sulphate, sodium sulphite and sodium hypo-sulphite. Sodium thio-sulphate was a moderate source among the inorganic sulphur compounds, while in organic sulphur compounds, *viz.*, Cystin and methionine, there was moderate sporulation. Thio-urea in case of *C. bertholletiae* supported moderate sporulation and poor in case of *C. echinulata.*

The above results showed that sulphate and sulphite were good sources while Cystin, methionine and thio-sulphate were found to be moderate source only. This clearly established that the two species of *Cunninghamella* investigated can utilize the oxidised sulphur source more readily than the reduced sulphur.

The results given in tables 2 and 3, also demonstrate that, in general, the sporulation was good, moderate, poor or nil according to the amount of growth obtained in various media.

SUMMARY

1. The comparative physiological study of the two species *Cunninghamella* viz., *Cunninghamella echinulata* and *Cunninghamella* bertholletiae was undertaken.

2. The temperature suitable for the best growth for both the species was 35°C (optimum temperature).

3. Amongst the inorganic sources, ammonium nitrate, sodium nitrite, proved to be good sources for the both the species, while potassium nitrate was moderate for *C. bertholletiae*. Asparagine and Acetamide were poor for *C. bertholletiae*, but good and moderate for *C. echinulata* respectively. Glutamic acid supported good growth of *C. echinulata*, but poor of *C. bertholletiae.*

4. Out of the inorganic sources of sulphur tried potassium sulphate, sodium-bi-sulphite, sodium sulphite and sodium hypo-sulphite were good sources for sulphur for both the fungi, while sodium-thio-sulphate proved to be mediocre for both.

5. The three organic sulphur compounds, cystin and methionine proved to be moderate sources for both the species but thio-urea was mediocre for *Cunninghamella bertholletiae*, and poor for *C. echinulata*.

6. The sporulation was best in ammonium nitrate amongst the nitrogen sources. In case of potassium sulphate, sodium bi-sulphite, sodium sulphite and sodium hypo-sulphite it was good, while in case of sodium thio-sulphate it was moderate. Out of the organic compounds cystin and methionine supported moderate sporulation, while thio-urea proved to be a moderate source for *Cunninghamella bertholletiae* and poor for *C. echinulata*.

7. The results given in tables 2 and 3, also demonstrate that, in general, the sporulation was good, mediocre, poor or nil according to the amount of growth obtained in various media.

LITERATURE CITED

1. Armstrong, G. M., 1921. Studies in the Physiology of Fungi XIV. *Ann. Mo. Bot. Gard.* VII, 237-80.
2. Bhargava, K. S., 1945. *Saprolegniaceae*, III. Nitrogen requirements. *Jour. Ind. Bot. Soc.*, **24**, 68-72.
3. ————1956. *Saprolegniaciae* II. Sulphur and Phosphorus requirements. *Proc. Ind. Acad. Sci.* **21**, No. 6, 344-349.
4. Bilgrami, K. S., 1956. Pathological and Physiological Studies of Some Fungi causing Leaf Spot Diseases, D. Phil. Thesis. Allahabad University.
5. Converse, R. H., 1953. *Mycologia*, **45**, 335-344.
6. Davis, B. D., 1955. *Advances in Enzymol.* Vol. **16**, 247-312.
7. Foster, J. W., 1949. Chemical activities of Fungi, Academic Press, Inc., New York.
8. Gottleib, D., 1946. *Arch. Bio. Chem.*, **9**, 341-351.
9. Gordon, M. A., 1950. *Mycologia*, **42**, 167-185.
10. Hagem, O., 1910. Mucorineen. H. Vidensk, Selak Skrift. *I. Math. Natur. Klasse*, **4**, 1-152.
11. Hawker, L. E., 1950. Physiology of Fungi, University of London Press.
12. Hesseltine, C. W., 1954. *Mycologia* **46**, 358-366.
13. Kumar, D., 1958. Physiological and Cytological Studies of some members of *Sphaeropsidaceae*. M.Sc. Thesis, Allahabad University (Unpublished).

*14. Leonian, L. H., and V. G. Lilly, 1938. *Phytopathology*, **28**, 531-548.

15. Lilly, V. G., and H. L. Barnett, 1951. Physiology of Fungi, McGraw Hill Book Company. Inc. New York—London.

*Original not consulted.

16. Lopatecki, L. E., and W. Newton, 1956. The nutrition of *Phytophthora* *Canad. Jour. Bot.* **34**, 750-757.

17. Mehrotra, B. S., 1949. Studies or some *Phytophthoras* and soil fungi. D. Phil. Thesis, Allahabad University.

18. Ram Dayal, 1959. Physiological & Cytological Studies on some aquatic fungi. D. Phil. Thesis, Allahabad University.

19. Ray Chaudhuri, S. P., 1942. A disease of Pigeon pea (*Cajanus cajan* (2) Mill Sp.) caused by *Diplodia cajani*. Spec. No.

20. Rai, P. P., 1951. Physiological & Cytological Studies on *Cunninghamella echinulata*. M.Sc. Thesis, Allahabad University (Unpublished).

21. Raizada, B. B. S., 1952. Some Cytological Studies of the asexual reproductive organs of *Cunninghamella echinulata* (Thaxter) and some Physiological Studies of *C. echinulata* and *C. elegans*, M.Sc. Thesis, University of Allahabad.

22. ————1957. Studies on Some Lower Fungi, D. Phil. Thesis, Allahabad University.

23. Saksena, R. K., 1958. *Proc. Nat. Acad. Sci. India* **10**, 1-13.

24. ————, S. K. Jain and S. M. N. Jafri, 1952. Sulphur & Nitrogen requirements. *Jour. Ind. Bot. Soc. XXXI* **4**, 281-286.

25. Steinberg, R. A., 1939. Synthetic nutrient Solutions II. *Bot Rev.*, **16**, 208-228.

26. Subramanium, C. V., & K. V. Srinivaspai, 1953. *Proc. Ind. Sci.* **37**, 149-151.

27. Tisadale, W. H., 1917. *Phytopathology*, **7**, 356-360.

28. Timnick, M. B., V. G. Lilly, and H. L. Barnett, 1951. *Mycologia*, **43**, 625-634.

29. Tandon, R. N., and K. S. Bilgrami, 1955. *Proc. Nat. Acad. Sci.* **24B**, 191-195.

30. Tandon, M. P., 1950. Physiological Studies of *Pestalotia malorum* and *Pestalotia psidii*, D. Phil. Thesis, Allahabad University.

31. Wolf, F. J., 1953. *Mycologia*, **45**, 516-522.

32. Yemn, E. W., 1954. Proc. Symposium Colston, Res. Soc. 51-66. New York Academic Press.

*33. Lemoigne, M., Monti guillon. P., and Desveaux, R. 1936. Utilisation des sels, d'hydroxylamine Par le Sterigmatocystis-nigra *Butl. Soc. Chim. Biol.*, **18**, 1291-1296.

*Original not consulted.

GROWTH OF EXCISED EMBRYOS OF WHEAT (*TRITICUM VULGARE* VAR. Pb. 591) IN DIFFERENT MEDIA WITH VARYING AGAR CONCENTRATION

By

REAYAT KHAN

Department of Botany, Aligarh Muslim University, Aligarh

and

L. F. RANDOLPH

Department of Botany, Cornell University, Ithaca, N.Y. (U.S.A.)

[Received on 12th March, 1960]

1. INTRODUCTION

Several basic nutrient media have been recommended by various authors for tissue, organ and embryo culture but their relative merits have not been adequately studied. In order to select a basic medium suitable for the culture of mature excised embryos of wheat, Randolph and Khan (1960) made comparative studies of five different media, namely, Knudson's Orchid Agar medium (a commercial mixture of the ingredients included in the Knudson's (1946) C medium, with the addition of 2% sucrose and agar to make a 1·5% mixture when diluted with water as specified by the manufacturer, the Difco Laboratories, Detroit, Michigan, U.S.A.) and the basic media of Randolph & Cox (1943; Randolph & Randolph, 1955), Rappaport (1954), Street (1954), Sheat, Fletcher and Street, 1959) and Nitsch (1951) (Table 1). The effect of varying the agar concentration in the first three of the above five media and in Knudson's C medium on the growth of embryos in culture was also studied. In the experiments reported here more comprehensive comparisons of different agar concentrations are made.

2. MATERIAL & METHODS

Grains of *Triticum vulgare* var Pb. 591 were used as experimental material. Samples obtained originally from the Economic Botanist, U.P. Government, Kanpur, were maintained locally by cultivation in experimental plots. Healthy grains were selected and surface sterilized with a saturated, aqueous solution of calcium hypochlorite for half an hour. The grains were then rinsed and soaked in sterile distilled water for about 15 hours. Excision and inoculations of the embryos were carried out in an ordinary room under a simple glass chamber. A spear-head needle was used for excising the embryo and transferring it to the culture tube. Before dissection of a grain, the fingers as well as the dissecting needle were sterilized with a 50% aqueous solution of "ST 37".

The culture tubes were kept in a culture room maintained under artificial light and temperature (between 18°C and 26°C).

TABLE 1

Components of the nutrient media studied

(mg/litre except where indicated otherwise)

	Knudson	Randolph & Cox	Street	Nitsch	Rappaport
Calcium Nitrate – $Ca(NO_3)_2.4H_2O$	1000	236·8	288	500	236·8
Potassium Nitrate – KNO_3		85	80	125	85
Potassium Chloride – KCl		65	65		65
Ferrous Sulphate – $FeSO_4.7H_2O$	25	2			
Calgon $(NaPO_3)_n$		10			10
Magnesium Sulphate $MgSO_4.7H_2O$	250	36	740	125	36
Ammonium Sulphate $(NH_4)_2SO_4$	500				
Potassium Dihydrogen Phosphate KH_2PO_4	250			125	
Manganese Sulphate – $MnSO_4.4H_2O$	7·5			3	0·5
Sodium Dihydrogen Phosphate NaH_2PO_4			21·5		
Sodium Sulphate – Na_2SO_4			453·4		
Copper Sulphate – $CuSO_4.5H_2O$			·02	0·025	
Boric Acid – H_3BO_3			1·5	0·5	
Zinc Sulphate – $ZnSO_4.7H_2O$			2·65	0·5	
Sulphuric Acid H_2SO_4				0·0005 ml	
Potassium Iodide – KI			0·75		
Molybdic Acid – H_2MoO_4			0·0017		
Ammonium Molybdate – $(NH_4)_6MoO_4.4H_2O$ (in place of Sodium Molybdate)				0·018	
Iron (in the form of FeEDTA in place of ferric sulphate)			1 p.p.m.		
Manganese Chloride – $MnCl_2.4H_2O$			6·0		
Ferric Citrate 1%				1 ml.	3 ml.
Sucrose	2%	2%	2%	2%	2%

Growth measurements were taken at the end of 5 days when the plants had grown sufficiently to fill the length of the culture tubes. The results were analysed statistically by means of analysis of variance and the *t* test. Differences were considered significant when the value of P was less then 0·05.

3. OBSERVATIONS

In a comparison of the growth of the root and shoot in five different media it was found that shoot growth was not significantly different in most cases. Root growth in Knudson's Orchid Agar medium at an agar concentration of 0·6% was inferior to the other four media which did not usually show significant differences among themselves. At an agar concentration of 1·5%, Knudson's Orchid Agar medium gave better result than Street's medium alone in only one out of three replicated experiments (see Randolph & Khan, 1960).

When a medium was prepared with agar (shreds) at a concentration of 1%, it was noted that the agar slants in the culture tubes sometimes cracked in the middle and the lower part of the medium slipped down. At 1·5% agar concentration, the m edium remained firm. However, measurements revealed, that root growth as well as shoot growth in a medium with 1·5% agar concentration was invariably poorer than in the same medium with 1% agar concentration except in the case of Knudson's Orchid Agar medium (Table 2). It was therefore considered necessary to find out if this difference was statistically significant. As Knudson's Orchid Agar medi um is a whole medium, the concentration of all its nutrient constituents is altered proportionately, when its agar concentration is altered. At lower agar concentrations, all the nutrients are also reduced in quantity; with increasing agar concentration, the nutrients become available in larger quantities. This explains the exceptional behaviour in this medium. The relevant growth measurements are presented in Table 2.

Results of statistical analysis of comparative growth values obtained with different pairs of media are shown in Table 3. Rappaport's medium at 1% and 1·5% agar concentrations did not give significantly different results either in root growth or shoot growth. Randolph-Cox medium with 1% agar concentration gave significantly better root growth than at 1·5% agar concentration but no significant difference in shoot growth was observed. Knudson's C medium at 1% and 1·5% agar concentrations did not give significantly different results either in root growth or shoot growth. Knudson's Orchid Agar medium was used at three different agar concentrations, viz., 0·6%, 1% and 1·5%. Knudson's Orchid Agar medium at 0·6% and 1% agar concentrations did not produce significantly different growth in either root or shoot. The same result was obtained in a comparison of Knudson's Orchid Agar medium at 1% and 1·5% agar concentrations. At 1·5% agar concentration, however, it gave significantly better root and shoot growths than at 0·6%.

TABLE 2

Growth (in m.m.) of embryo cultured seedlings in four media, viz., Knudson's C medium (KC), Knudson's Orchid Agar (KO) and the media of Randolph-Cox (RC) and Rappaport (Rp) with different agar concentrations (figures in parentheses).
S.D. = Standard Deviation.

Nutrient media with agar concentration	Root growth Mean & S.D.	Shoot growth Mean & S.D.
KC (1·0)	120·1 ± 4·5	73·6 ± 4·0
KC (1·5)	118·6 ± 7·0	63·5 ± 3·7
KO (0·6)	86·1 ± 4·2	47·4 ± 5·4
KO (1·0)	97·0 ± 6·4	59·2 ± 3·1
KO (1·5)	111·1 ± 5·8	61·3 ± 2·0
RC (1·0)	165·0 ± 7·0	64·6 ± 4·8
RC (1·5)	131·3 ± 8·0	50·6 ± 4·9
Rp (1·0)	143·9 ± 6·4	67·1 ± 2·7
Rp (1·5)	123·4 ± 9·1	56·0 ± 6·7

TABLE 3

Comparison of root and shoot growth of embryo cultured seedlings in different media based upon the data given in Table 2. × = Not significantly different. Other symbols and figures as in Table 2.

Nutrient media with agar concentration	Root growth	Shoot growth
Rp (1·0) & Rp (1·5)	×	×
Rp (1·0) & RC (1·0)	RC (1·0) superior	×
Rp (1·0) & RC (1·5)	×	Rp (1·0) superior
Rp (1·0) & KC (1·0)	Rp (1·0) superior	×
Rp (1·0) & KC (1·5)	Rp (1·0) superior	×
Rp (1·0) & KO (0·6)	Rp (1·0) superior	Rp (1·0) superior
Rp (1·0) & KO (1·0)	Rp (1·0) superior	×
Rp (1·0) & KO (1·5)	Rp (1·0) superior	×
Rp (1·5) & RC (1·0)	RC (1·0) superior	×
Rp (1·5) & RC (1·5)	×	×
Rp (1·5) & KC (1·0)	×	×
Rp (1·5) & KC (1·5)	×	×
Rp (1·5) & KO (0 6)	Rp (1·5) superior	×
Rp (1·5) & KO (1·0)	Rp (1·5) superior	×
Rp (1 5) & KO (1·5)	×	×
RC (1·0) & RC (1·5)	RC (1·0) superior	×
RC (1·0) & KC (1·0)	RC (1·0) superior	×
RC (1·0) & KC (1·5)	RC (1·0) superior	×
RC (1 0) & KO (0·6)	RC (1·0) superior	RC (1·0) superior
RC (1·0) & KO (1·0)	RC (1·0) superior	×
RC (1·0) & KO (1·5)	RC (1·0) superior	×
RC (1·5) & KC (1·0)	×	KC (1·0) superior
RC (1 5) & KC (1·5)	×	KC (1·5) superior
RC (1·5) & KO (0·6)	RC (1·5) superior	×
RC (1·5) & KO (1·0)	RC (1·5) superior	×
RC (1·5) & KO (1·5)	RC (1·5) superior	KO (1·5) superior
KC (1·0) & KC (1·5)	×	×
KC (1·0) & KO (0·6)	KC (1·0) superior	KC (1·0) superior
KO (1·0) & KO (1·0)	KC (1·0) superior	KC (1·0) superior
KC (1·0) & KO (1·5)	×	KC (1·0) superior
KC (1·5) & KO (0·6)	KC (1·5) superior	KC (1·5) superior
KC (1·5) & KO (1·0)	KC (1·5) superior	×
KC (1·5) & KO (1·5)	×	×
KO (0·6) & KO (1·0)	×	×
KO (0·6) & KO (1·5)	KO (1·5) superior	KO (1·5) superior
KO (1·0) & KO (1·5)	×	×

When Rappaport's and Randolph-Cox media were compared at 1% agar concentration, Randolph-Cox proved to be superior for root growth while shoot growth showed no significant difference. When the two media were compared at 1·5% agar concentration there was no significant difference either in root or shoot growth. When Rappaport's medium was compared with Knudson's C medium at 1% agar concentration, Rappaport's medium gave better root growth but there was no significant difference in shoot growth. When the two media were compared at 1·5% agar concentration, there was no significant difference in root or shoot growth. In a comparison of Rappaport's medium and Knudson's Orchid Agar medium at 1% agar concentration, Rappaport's medium gave better root growth but shoot growth revealed no significant difference. At 1·5% agar concentration the two media showed no significant difference in either root growth or shoot growth.

Comparing Randolph-Cox and Knudson's C media at 1% agar concentration, Randolph-Cox gave better root growth but no significant difference was noted in shoot growth. At 1·5% agar concentration, root growth showed no significant difference but shoot growth was better with Knudson's C medium. Comparing Randolph-Cox medium and Knudson's Orchid Agar medium at 1% agar concentration, Randolph-Cox gave better root growth but shoot growth exhibited no significant difference. At 1·5% agar concentration, root growth was better with Randolph-Cox while shoot growth was better with Knudson's Orchid Agar medium. When Knudson's C medium and Knudson's Orchid Agar medium were compared at 1% agar concentration, the former proved superior both for root growth and shoot growth. At 1·5% agar concentration the two media exhibited no significant difference either in root growth or shoot growth.

In Table 3 the column presenting comparisons of root growth shows that out of 36 comparisons made, the agar concentration of 1·5% gave better results in 8 cases while 1% agar cencentration gave better results in 15 cases, the results showing no significant difference in the remaining cases. The column presenting comparisons of shoot growth at different agar concentrations shows that out of 36 comparisons made, the agar concentration of 1·5% gave better results in four cases while 1% agar concentration gave better results in 7 cases, the results showing no significant differences in the remaining cases. Apparently the root system developing from wheat embryos in culture is more selective in its requirements than the shoot.

4. SUMMARY

Growth of excised mature embryos of wheat was studied in five different media, viz., Knudson's Orchid Agar medium and the basic media of Rappaport, Randolph-Cox, Street and Nitsch. In the first three of these media and Knudson's C medium, comparisons involving the agar concentration of the media were made.

In the comparison of growth in the five media, shoot growth did not exhibit significant differences in the majority of cases. Root growth in Knudson's Orchid Agar medium diluted to obtain an agar concentration of 0·6% was inferior to all other media which did not usually show significant differences among themselves. At an agar concentration of 1·5% Knudson's Orchid Agar medium gave better root growth only in comparison to Street's medium in one out of three experiments.

Comparisons of the same medium at 1% and 1·5% agar concentrations showed significant difference only in the case of Randolph-Cox medium. This medium at 1% agar concentration gave better root growth than at 1·5%, but no significant difference was noted in the case of shoot growth.

In comparisons of media at the same agar concentration, shoot growth did not show a significant difference in the majority of cases. When two different media were compared at 1% agar concentration, root growth invariably exhibited significant difference, one of the two media giving better result than the other. But when the same media were compared at 1·5% agar concentration, root growth exhibited significant difference in only one out of six cases. This may suggest that, in order to enable the finer distinctions between media to express themselves, comparisons should be made at relatively low agar concentrations.

The root system of wheat seedlings produced by embryos in artificial culture seems to be more selective in its requirements than the shoot.

5. ACKNOWLEDGEMENT

This work was carried out when the second author was in residence at the Aligarh Muslim University as a visiting Professor in Botany and Fulbright Scholar. The research facilities made available by the University are gratefully acknowledged. The authors are indebted to Mr. Z. H. Zaidi and Mr. R. S. Yajnik for assistance.

6. LITERATURE CITED

Knudson, L., 1946, A new nutrient solution for the germination of Orchid seeds. *Amer., Orchid Soc., Bull.,* **15**: 214-217.

Nitsch, J. P., 1951, Growth and development in vitro of excised ovaries. *Amer., J. Bot.* **38**: 566-577.

Randolph, L. F., & Cox, L. G., 1943, Factors influencing the germination of iris seed and the relation of inhibiting substances to embryo dormancy. *Proc. Amer. Soc. Hort. Sci*, **43**: 284-300.

Randolph, L. F. & Khan, R., 1960, Growth response of excised mature embryos of iris and wheat to different culture media. *Phytomorphology*, **10**: 43:49.

Randolph, L. F. & Randolph, F. R., 1955, Embryo culture of iris seed. *Amer. Iris Soc. Bull.* **139**: 2-12.

Rappaport, J., 1954, In vitro culture of plant embryos and factors controlling their growth. *Bot. Rev.* **20**: 201-225.

Sheat, D. E. G., Fletcher, B. H. & Street, H. E., Studies on the growth of excised roots VIII. The growth of excised tomato roots supplied with various inorganic sources of nitrogen. *New Phytol.* **58**: 128-154.

Street, H. E., 1954, Growing roots without plants. *Discovery* **15**: 286-292.

STUDIES ON A NEW SPECIES OF THE GENUS PEGOSOMUM RATZ, 1903 (TREMATODA: ECHINOSTOMATIDAE)

By

J. N. SAKSENA*

Department of Zoology, M. L. B. College, Gwalior

[Received 20th June, 1960]

Two worms were collected from the bile duct of the cattle egrett, *Bubulcus. ibis* shot near Raipur (M. P.) in the month of August, 1956. They belong to the genus *Pegosomum* Ratz, 1903. Seven species of the genus are known from the world. They are *P. asperum* (Wright. 1876) Ratz, 1903, ; *P. saginatum* (Ratz, 1898) Ratz, 1903; *P. spiniferum* Ratz, 1903 ; *P. bubulcum* Tubangui and Masilungari, 1935 ; *P. skrjabini* Shakhtakhtinskaya, 1949; *P. petrowi* Kurashviti, 1949 and *P. egretti* Srivastava, 1957. Only one species—*P. egretti* is known from India. This new species will form the second species of the genus *Pegosomum* from India.

Pegosomum indicum n. sp. (Text fig. 1)

The body of the worm is spindle shaped, measuring 3·7-4·1 mm. in length and 1·0 – 1·1 mm. in breadth at the region of the acetabulum. The cephalic collar is poorly developed measuring 0·18 mm. in length and 0·32 – 0·34 mm. in breadth. The collar lappets do not meet ventrally. The whole body is covered with small backwardly directed spines while at the collar region the spines form a coronet. In the glycerine cleared specimen the body spines are clearly seen and they are bigger than the collar spines. The body spines at the level of the ovary measures. 0·021 mm. in length and 0·006 mm. in breadth, while the biggest angular spine of the collar measures 0·015 mm. in length and 0·003 mm. in breadth. The ventral corner group of angular spines on each side consists of four bluntly pointed spines which are arranged in a single row. It is not possible to count the total number of collar spines forming the coronet as they are poorly developed, leathery and small. They get mixed up with the musculature of the collar.

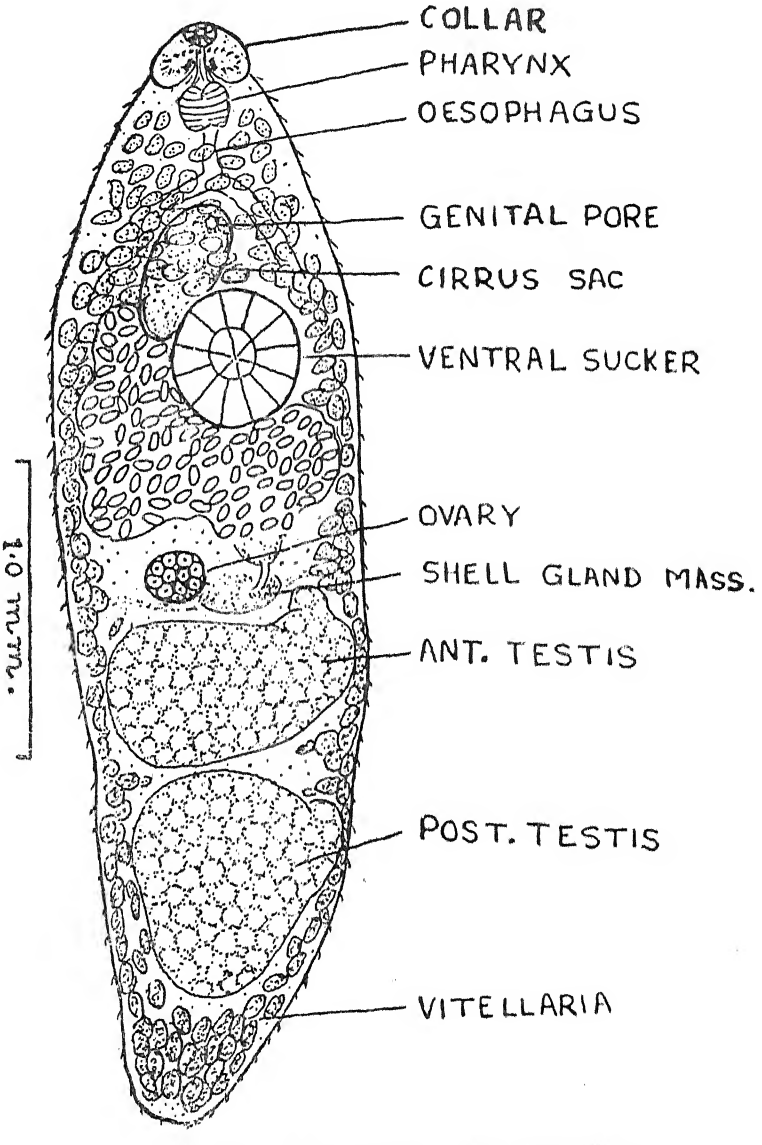
The oral sucker is poorly developed. It is triangular in shape measuring 0·08 – 0·1 mm. in length and 0·11 mm. in breadth. The ventral sucker is situated at a distance of 0·9 – 0·99 mm. from the anterior end. It is almost spherical measuring 0·45 - 0·49 mm. in diameter. The prepharynx is 0·1 mm. long and 0·03 mm. broad. The pharynx is well developed and muscular, measuring 0·17 – 0·18 × 0·13 – 0·14 mm. in size. The oesophagus is 0·18 – 0·21 mm. long. The intestinal bifurcation lies at the distance of 0·52 – 0·57 mm. from the anterior end. The intestinal caeca extend upto the posterior end of the body.

The excretory pore lies at the posterior end of the body. It leads into a Y-shaped excretory bladder. The genital pore is situated some distance below the intestinal bifurcation in the median line.

The gonads are located in the posterior half of the body. The anterior testis, situated at a distance of 1·99 – 2·3 mm. from the anterior end, is boat shaped, transversely placed covering nearly the whole breadth. It measures 0·76 – 0·88 × 0·42 – 0·49 mm. size. The posterior testis is triangular in shape measuring 0·70 –

*Present Address—Assistant Professor in Zoology, Mahakoshal Mahavidyalaya, Jabalpur.

0·74 × 0·7 – 71 mm. The distance between the two testes is 0·01 mm. The cirrus sac measures 0·45 – 0·49 × 0·22 – 0·27 mm. It lies anterior to the acetabulum covering the area upto half the level of the acetabulum on its right side. It encloses vesicula seminalis, pars prostatica and the muscular cirrus.



Text Fig. I. *Pegosomum indicum* n. sp.-Ventral View.

The ovary is oval in shape, lying above the anterior testis slightly towards the right side of the median line. It measures 0·21 – 0·25 × 0·17 – 0·18 mm. The shell gland mass lies on the left side of the ovary. The receptaculum seminis is absent. The uterus arises from the anterolateral margin of the shell gland mass and its coils are transversely placed in the intercaecal area between the acetabulum and the ovary.

The vitelline glands consist of small follicles extending from the middle level of the pharynx to the posterior end of the body. From the pharynx to the anterior level of the acetabulum the vitellaria unite in the median plane forming a continuous zone. Similarly below the posterior testis they are densely crowded and meet with each other. In the remaining portions the vitellaria lie in the lateral sides of the body, covering the intestinal caeca completely. The transverse vitelline ducts are located in front of the anterior testis and meet the shell gland by a small common duct. The eggs are present in large numbers. They are oval in shape, measuring 0·072 × 0·057 mm. in size.

DISCUSSION

Pegosomum indicum n. sp. differs from all the species of the genus *Pegosomum* in possession of small size of the body, poorly developed collar spines, comparatively large number of eggs and their small size. It differs from *P. bubulcum* and *P. egretti* in the posterior extension of vitellaria. The author is unable to get the references of the Russian species *P. skrjabini* and *P. petrowi*. Hence they are excluded from the discussion.

Key to the species of the genus *Pegosomum*.

A	Vitellaria do not extend behind testes	...	B
	Vitellaria extend upto posterior end of body	...	C
B	Collar spines 25 in two alternating rows, vitellaria extending from middle level of oesophagus	...	*P. egretti.*
	Collar spines 27 in one row, vitellaria extending from level of pharynx	...	*P. bubulcum.*
C	Body large, 8-25 mm. long, collar spines well developed, vitellaria do not form a crowd below testes	...	D
	Body small, 3-5 mm. long, collar spines poorly developed, vitellaria meet behind testes to form a crowd.	...	*P. indicum* n. sp.
D	Vitellaria tending to fill up anterolateral region of body, body 9-10 mm. long, 27 collar spines, eggs large – 0·119 × 0·085 mm. in size		*P. spiniferum.*
	Vitellaria do not fill anterolateral region of body		E
E	Body 14-24 mm. long, 20-21 collar spines, eggs few large – 0·096 – 0·13 × 0·069 – 0·085 mm. in size	...	*P. saginatum.*
	Body 8·19 mm. long, 27 collar spines, eggs comparatively small – 0·096 × 0·069 mm. in size	...	*P. asperum.*

ACKNOWLEDGEMENT

The author is grateful to Dr. S. S. Saxena, and Dr. Shamacharan for providing the research facilities in the department. Thanks are also due to Dr. R. N. Singh, Department of Zoology, Science College, Raipur, for taking keen interest in this work.

REFERENCES

Dawes, Ben, 1946. "The trematoda." Camb. Univ. Press.

Ratz, 1903. Un genre nouv. de Fasc., in *Ann. Hist. nat. Mus. Hung.* Budapest, Vol. I, 427—431.

Shakhtakhtinskaya, Z., 1949. (*Pegosomum skrjabini* sp. nov. parasite of birds) in Russian. *Trud. gel'mint.* lab. 2, 1949: 87-90.

Srivastava, O. N. 1957. A new species of *Pegosomum*, an Echinostome (Trematoda) from cattle egrett. *Proc. Nat. Acad. Sc., India.* Vol XXVII, Sec. B, Part III : 129-134.

Tubangui, M. A. and Masilungari, U. A., 1935. *Pegosomum Bubulcum* sp. n. from cattle egrett. *Philipp J. Sc.* Manila. 58.

Wright, 1879. *Distomum asperum*, contrib, to Americ. Helminth., in *Proc. canad. Inst.*, Vol. 1, P. 6, No. 2 tab. 1 fig. 3

Yamaguti, S. 1958. Systema Helminthum, Vol. 1. The digenetic trematodes of vetebrates in two parts. Interscience Publishers, INC. NEW YORK.

UTILIZATION OF DIFFERENT CARBON COMPOUNDS BY TWO SPECIES OF *CUNNINGHAMELLA*

By

R. K. SAKSENA *and* A. K. SARBHOY

Botany Department, University of Allahabad, Allahabad

[Received on 7th July, 1960]

INTRODUCTION

The role of carbon in fungal nutrition has now been thoroughly investigated. It is a recognised fact that carbon occupies one of the premier positions amongst the various essential elements required by living organisms including fungi, which, generally, prefer carbonydrates as their important food. It has now been accepted that all fungi do not utilize the same carbohydrates equally well. This only depends on the ability of the fungus and the chemical constituents of the compounds. Glucose, fructose and sucrose serve as some of the best sources of carbon for many fungi (Young and Bennet 1922; Leonian 1924). There are certain fungi which do not utilize some of these for example *Leptomitus lacteus* (Schade, 1940, Schade and Thimann, 1940) does not employ glucose, fructose, galactose and sucrose.

Studies on the carbon requirements of the various members of the order Mucorales have been done in the past few years and many workers have included species of *Mucor, Phycomyces* and *Rhizopus* in their investigations. It has also been reported by many workers that closely allied species of the same genus may differ considerably in the choice of a given sugar. Volkonsky (1934) observed that *Phytophthora parasitica* utilized raffinose rapidily while another species used the same sugar slowly. Herrick (1940) found similar results with some isolates of *Stereum gausapatum.* Steinberg (1939) reported that D-glucose, D-fructose, D-mannose, L-sorbose and sucrose are equally utilizable by *Aspergillus niger* which, on the other hand, used poorly D-galactose, lactose, glycerol and mannitol. Margolin (1942) found lactose to be a poor source of carbon for growth of many fungi included in his studies. Barnett and Lilly (1950) reported that glucose was the best source of carbon for the growth of one isolate of *Choanephora cucurbitarum* but it was poor or mediocre for the other. Bhargava (1945) found glucose, fructose, maltose and starch to be good carbon sources for *Achlya sp., Brevilegnia gracilis, Isoachlya sp., Saprolegnia delica* and *S. monoica.* Saksena (1940) also reported similar results regarding the utilization of dextrose, maltose, sucrose and starch for some species of *Pythium.*

It has been observed by many workers that generally carbon sources which support good growth do not permit good sporulation, *i. e.*, the carbon requirement for growth and sporulation differ (Lilly and Barnett, 1951, p. 324). Barnett and Lilly (1950) found sucrose and lactose to be good or mediocre sources for the sporulation and poor for the growth of an isolate of *Choanephora cucurbitarum.*

The present investigation was undertaken to determine the role of various carbon compounds on the growth and sporulation of *C. echinulata.* Thaxter and *C. bertholletiae* Stadel. The morphological changes in the two isolates were also determined under these conditions.

MATERIAL AND METHODS

Cunninghamella echinulata Thaxter and *Cunninghamella bertholletiae* Stadel were isolated by the authors from a soil sample obtained from the Agriculture Farm, Allahabad University.

For experiments on carbon requirements the 'SMA' medium (Hesseltine; 1954) without the carbon source (*i. e.* minus dextrose) acted as the basal medium. (Asparagine 2 gms., Potassium acid phosphate 0·5 gm., Magnessium sulphate, $7H_2O$, 0·25 gm., Thiamine chloride 0·5 m. gm., Distilled water 1,000 c. c.). Various carbon compounds viz., (monosaccharides (pentoses, hexoses), disaccharides, trisaccharides, polysaccharides, alcohols and organic acids (trihydric and tetrahydric) were added singly to the basal medium in amounts calculated to furnish 1,600 m. gms. of carbon per litre. The pH of the medium was adjusted to 6·8 before autoclaving.

25 c. c. of liquid media were poured in 150 c. c. Erlenmeyer Pyrex flasks, which were autoclaved at 15 lbs. pressure for 15 minutes except those containing the disaccharides and trisaccharides, which were steamed at no pressure for 15 minutes for three consecutive days to avoid hydrolysis. These flasks were inoculated with the inoculum obtained from the margin of actively growing colonies of one day old cultures on the above medium. The inoculated flasks were then incubated for 15 days at room temperature (25°C mean). After an interval of every three days one flask was taken out from each set of different carbon compounds for examination of sporulation and morphological changes. Eight flasks of each medium were inoculated for this experiment.

After the incubation period, the contents of the flasks were filtered, fungal mats were washed, dried and weighed for the determination of dry weights. The filtrate in each case was used for the determination of change in pH of the medium.

In the second part a chromatographic study was made to determine the pathway of the utilization of fructose (monosaccharide) and sucrose (disaccharide) by *Cunninghamella bertholetiae*. Before starting the experiment it was ascertained chromatographically that hydrolysis of sugars had not taken place during steaming. The flasks containing the media were inoculated with actively growing fungi on 'SMA' medium daily for 15 days at a fixed time ($\pm$ 15 minutes) by agar disc method. After the incubation period the contents of the flasks were filtered, washed, dried and weighed for the determination of dry weight. The filtrate in each case was used for chromatographic studies.

The technique used by Tandon and Bilgrami (1956) for the chromatographic analysis of the two media was employed. A circular piece of Whatman's filter paper No 1 having a diameter of 26 c. m. with radial cuts according to the number of days was used. From the filtrate, drops of 0·5 ml. were placed at the position located for this purpose (No. 1, 2 to 15). Equal volumes of drops of known solutions were also placed on the chromatograms (position K) to facilitate the identification of bands. Drops from the control solution were also put for the reference (position C). The chromatograms were run with n-butanol : acetic acid : water (4 : 1 : 5) as solvent. They were then sprayed by a mixture of aniline; 5 vols. of 4% diphenylamine and one vol. of phosphoric acid. The bands were developed by placing the chromato-grams in an electric oven at 110°C for one to 2 minutes. On the basis of these bands the average Rf of the various sugars have been calculated.

TABLE 1. Showing the average dry weight and sporulation of *C. echinulata* on different carbon sources and the corresponding change in pH value of the media.

Carbon compounds	Dry weight in m. gms.	Sporulation	pH
1. Xylose	29·0	poor	5·8
2. Glucose	48·7	good	4·3
3. Fructose	40·0	good	3·9
4. Maltose	79·7	excellent	3·2
5. Sucrose	30·0	poor	3·8
6. Lactose	39·0	good	6·6
7. Raffinose	40·7	good	3·9
8. Starch (soluble)	28·7	poor	3·4
9. Dextrin	43·3	good	3·2
10. Glycerol	52·0	excellent	2·5
11. Mannitol	15·3	poor	3·8
12. Malic acid	2·7	poor	6·3
13. Tartaric acid	3·7	poor	6·5
14. Control (no carbon)	0·0	nil	6·2

Average Mean of the dry weight = 32·4

Summary of the dry weight results and conclusions at 1 % level of P.

Treatments	- highly significant
Replicates	- non-significant
S. E.	- 0·491
C. D. at 1 % level	- ± 1·92

Dry weight results :

Carbon compounds : Maltose > Glycerol > Glucose > Dextrin >
Dry weight in m. gms. 79·7 52·0 48·7 43·3

Raffinose 40·7, Fructose 40·0, Lactose 39·0 > Sucrose 30·0, Xylose 29·0, Starch 28·7 >

Mannitol > Tartaric acid, Malic acid > Control
15·3 3·7 2·7 0·0

TABLE 2. Showing the average dry weight and sporulation of *C. bertholletiae* on different carbon sources and the changes in pH values of the media.

Carbon Compounds	Dry weight in m. gms.	Sporulation	pH
1. Xylose	6·6	poor	5·2
2. Glucose	88·6	good	4·3
3. Fructose	50·3	moderate	5·3
4. Maltose	132·0	excellent	4·2
5. Sucrose	83·6	good	5·2
6. Lactose	17·3	poor	6·5
7. Raffinose	31·6	poor	5·2
8. Starch	148·3	excellent	4·2
9. Dextrin	169·3	excellent	4·0
10. Glycerol	36·6	poor	5·0
11. Mannitol	14·0	poor	5·2
12. Malic acid	14·3	poor	5·5
13. Tartaric acid	7·3	poor	6·2
14. Control (no carbon)	0·0	nil	6·4

Average mean of the dry weight = 57·22

Summary of dry weight results and conclusions at 1% level of P.

Treatments	- highly significant
Replicates	- non-significant
S. E.	- 3·452
C. D. at 1% level	- ± 13·53

Dry weight results :

Carbon compounds : Dextrin > Starch > Maltose > Glucose
Dry weight in m. gms. 169·3 148·3 132·0 88·6

Sucrose > Fructose Glycerol Raffinose > Lactose Malic acid Mannitol
83·6 50·3 36·6 31·6 17·3 14·3 14·0

Tartaric acid Xylose > Control
7·3 6·6 0·0

A critical study of the above tables indicates that the pentose sugar, xylose supported moderate growth of *C. echinulata* while it was poor source for *C. bertholletiae.* Out of the two hexoses, glucose supported good growth of both the species but fructose was a good source only for *C. echinulata* and mediocre for *C. bertholletiae.*

Amongst the disaccharides, maltose supported good growth for both the species. There was good growth of *C. echinulata* on lactose but poor *C. bertholletiae.* Sucrose supported good growth of *C. bertholletiae* and mediocre for *C. echinulata.*

Out of the two polysaccharides viz., starch and dextrin, the latter supported good growth of both the species, while soluble starch was good for *C. bertholletiae* and moderate for *C. echinulata.* Glycerol was found to be good for *C. echinulata* and poor for *C. bertholletiae.* Mannitol, on the other hand, was poor for both the species.

Organic acids have been found to be poor sources of carbon for the two species.

In absence of any definite method for ascertaining the degree of sporulation in the two species, only visual observations served for estimating spore production. Timnick *et al* (1951) also used the same method.

Pentose sugars supported poor sporulation for both the species.

Sporulation was excellent on maltose and glucose in case of both the organisms, good on fructose in case of *C. echinulata* and moderate for *C. bertholletiae.*

Among the disaccharides sucrose supported poor sporulation for *C. echinulata* but was good for *C. bertholletiae,* while lactose was good for *C. echinulata* and poor for *C. bertholletiae.*

Raffinose was found good for sporulation in *C. echinulata* while it was poor for *C. bertholletiae.*

Starch and dextrin also supported good sporulation of *C. bertholletiae* but only moderate in *C. echinulata.*

Glycerol was excellent for sporulation of *C. echinulata,* but poor for *C. bertholletiae* ; mannitol supported poor sporulation in both the species.

The different organic acids employed caused poor sporulation of the two species.

In almost all the cases the pH of the medium became acidic with the growth of the fungi but with lactose only a slight difference from the initial pH was noticed.

Assimilation rate of mono and di- saccharides :

Fructose, the only mono-saccharide used in the present study, was present in the medium till the 15th day. It was clear that it could not be cosumed by the organism within the specified period. However, the decrease in the intensity of the band shows poor untilization of this sugar.

TABLE 3. Results obtained with the Fructose and Sucrose (the hydrolytic product).

	DAYS														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
C. bertholletiae															
Presence of fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Dry weight in m. gm.	1·2	3·3	6·0	7·3	8·0	10·6	14·6	15·3	16·0	18·0	19·3	23·0	23·0	30·0	32·0
C. bertholletiae															
Presence of sucrose	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
Presence of glucose	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
Presence of fructose	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
Dry weight in m. gms.	0·9	1·5	3·0	3·0	3·0	4·0	4·0	4·0	5·0	6·0	8·0	10·2	12·0	13·2	17·2

+ denotes the presence of Sugar.

− denotes the absence of Sugar.

Sucrose (Rf. 0·50) breaks up into glucose and fructose. The chromatographic analysis of the medium revealed that it was hydrolysed on the 3rd day and on the 5th day it (sucrose) had totally disappeared from the medium leaving glucose and fructose, which persisted in the medium till the 15th day; later on the experiment was run for 22 days and even then these sugars were persent in the medium. This shows the poor utilization of these two mono-saccharides.

DISCUSSION

Utilization of various carbon compounds depends on the ability of fungi either to assimilate them or to convert complex carbon compounds into forms that can be used directly. In the latter process the enzymes associated with the organisms play an important role. The carbon compounds which can be assimilated more readily or can be oxidised by the organism with the least expenditure of energy constitute the food of first choice.

The results obtained by the authors show that there is a substantial difference in the ability of the two species for utilizing different sugars.

Xylose, the pentose sugar, has been found to be a poor source of carbon for *C. bertholletiae* while it was moderate for *C. echinulata*. This has also been the observation of a number of other workers (Mehrotra 1951 and Raizada 1957).

Sucrose has been found to be good for *C. bertholletiae* and poor for *C. echinulata*. Lilly and Barnett (1953) also found sucrose to be a poor source of carbon for *Monoascus purpurea*, *M. ramanianus* and *Sordaria fimicola*.

Reverse has been the case with lactose, which has been observed to be good for *C. echinulata* and poor for *C. bertholletiae*. Margolin (1942) also found that *M. ramannianus* utilizes lactose poorly.

There has been a marked difference in the ability of the two species to utilize raffinose. It has been found to be a good source of carbon for *C. echinulata* (Lilly and Barnett 1953), *Absidia cylindrospora*, *C. echinulata* and *Rhizopus sontii* (*Raizada*, 1957), but it is poor for *Pythium* spp. (Saksena, 1940), for *Phytophthora* (Mehrotra, 1951) and and *Mucor hiemalis* and *M. rouxii* (Raizada, 1957).

Excellent growth of *C. bertholletiae* on starch and only moderate growth of *C. echinulata* show that in *C. bertholletiae* amylase enzyme is more active than in *C. echinulata*.

Sugar alcohols have generally been reported as poor sources of carbon for a number of fungi. Here glycerol was found to be good for *C. echinulata* but poor for *C. bertholletiae*. These results are in agreement with those of Kendrick and Walker (1948) for *Colletotrichum phomoides*, and of Kumar (1958) for *Macrophomina phaseoli*, *Botryodiplodia theobromae* and *Diplodia cajani*. Mannitol was poor for both the species as has also been found by Mehrotra (1951) for 10 species of *Phytophthora* and also by Raizada (1952) for *C. echinulata* and *C. elegans*.

Organic acids have been found to be poor sources of carbon for the two species studied by the authors. Same has been the findings of Raizada (1957) for *M. fragilis* and *Choanephora cucurbitarum*.

Sporulation depends upon a number of factors. Generally, it is reported that the carbon sources so far tried are not equally suitable for the sporulation of the

different organisms. In the present studies the carbon sources employed also behaved differently.

Lilly and Barnett (1951) reported that the best source of carbon for sporulation was not the same which yielded the maximum growth. In the present study with the fungi tested by the authors, good sporulation was accompanied with good growth and this is in agreement with the results obtained by Raizada (1957) for *Absidia cylindrospara, C. echinulata, Rhizopus sontii, Mucor hiemalis.* Sporulation was excellent in both the species on maltose and glucose. Glycerol and raffinose could support good sporulation of *C. echinulata* but not of *C. bertholletiae.* On the other hand, sucrose, starch and dextrin supported good sporulation of *C. bertholletiae* and only moderate of *C. echinulata.* The organic acids supported poor sporulation of the two species.

In all the cases growth was accompanied with acidification of the media, but with lactose only a slight difference in pH was noticed though there was good and poor growth of *C. echinulata* and *C. bertholletiae* respectively.

The chromatographic studies made by the authors with sucrose and fructose confirm the earlier results obtained with these sugars under carbon requirement studies.

It has been found that the fungi under investigation are not capable of utilizing sucrose as such but they split it into glucose and fructose. The latter sugars are utilized by these fungi. These results are in agreement with those of Raizada (1957) for *Absidia cylindrospora* and *Cunninghamella echinulata.*

SUMMARY

1. Carbon requirements of *Cunninghamella echinulata* and *Cunninghamella bertholletiae* were investigated.

2. Amongst the monosaccharides, xylose was utilized by the two species but not more effectively. All hexoses were most favourably used by the two species.

3. Amongst the disaccharides, maltose proved to be the best source of carbon followed by sucrose. Lactose was good for *C. echinulata* but poor for *C. bertholletiae.*

4. Raffinose also reacted favourably for *C. echinulata* but poorly for *C. bertholletiae.*

5. Soluble starch proved to be a moderate source for *C. echinulata* but good for *C. bertholletiae.* Dextrin, in general, proved to be an excellent source for both the species.

6. The two alcohols behaved differently, glycerol was comparatively prefered over mannitol.

7. Of the organic acids, malic acid supported poor growth of *C. bertholletiae,* and none at all in the case of *C. eehinulata.* Reverse was the case with tartaric acid.

8. In most cases good sporulation was accompanied with good or fair growth.

9. The final pH of the media showed a tendency to drift towards the acidic side. With lactose only a slight difference from the initial pH was noticed. This needs further investigation.

10. Different responses of the two species of the same genus *Cunninghamella* to different carbon sources can be utilized as a good subsidiary character for distinguishing the two species.

11. The chromatographic studies made by the authors with sucrose and fructose, showed that these fungi are not capable of utilizing sucrose as such but they split it into glucose and fructose. The latter sugars are utilized by these fungi.

12. The results given in tables 1, 2 and 3 also demonstrate that, in general, the sporulation was excellent, good, mediocre, poor or nil according to the amount of growth obtained in various media.

LITERATURE CITED

1. Bhargava K. S., 1945, *Saprolegniaceae* IV, Carbohydrate requirement, *Lloydia* **8** : 60-68
2. Barnett, H. L., and V. G. Lilly, 1950, *Phytopathology*, **40** : 80-89.
3. Bilgrami, K. S., 1956, D. Phil. Thesis, University of Allahabad.
4. Hesseltine, C. W., 1954 *Mycologia* **46** : 358-366.
5. Herrick, J. A., 1940, *Ohio Jour. Sci.* **40** : 123-129.
6. Kumar, D., 1958, M. Sc. Thesis, University of Allahabad (Unpublished).
7. Leonian, L. N., 1924, *Amer., Jour. Bot.* **11** : 19-25.
8. Lilly, V. G., and H. L. Barnett, 1953, *Bull.* 352T. *West Virginia, Uni. Agri. Expt. Station.*
9. Mehrotra, B. S., 1949, D. Phil. Thesis, University of Allahabad.
10. Margolin, A. S., 1942, Thesis, West Virginia University.
11. Raizada, B. B. S., 1952, M. Sc. Thesis, University of Allahabad (Unpublished).
12. ————1957, D. Phil. Thesis, University of Allahabad.
13. Saksena, R. K., 1940, *Proc. Nat. Acad. Sci.* India, **10** : 1-13.
14. Steinberg, R. A., 1939, *Bot. Rev.* **5** : 327-350.
15. Schade, A. L., 1940, *Amer. Jour. Bot*, **27** : 376-384.
16. Schade. A. L., and K. V. Thimann, 1940, *Amer. Jour. Bot.* **27**, 659-670.

*17. Volkonsky, 1922, *Ann. Inst. Pasteur*, Paris, **50** : 703-730.

*18. ————1934, *Ann, Ins. Pasteur*, Paris, 52 : 76-101.

19. Young, H. C., and Bennet, C. W., 1922, *Amer. Jour. Bot.* **9** : 459-469.

* Originals not consulted.

EDITORIAL BOARD

1. Dr. M. S. Randhwa, New Delhi (*Chairman*)
2. Prof. P. S. Gill, Aligarh
3. Prof. K. Banerji, Calcutta
4. Prof. Ram Behari, Delhi
5. Prof. P. L. Srivastava, Allahabad
6. Prof. S. Ghosh, Allahabad
7. Prof. A. K. Bhattacharya, Sagar
8. Prof. N. R. Dhar, Allahabad
9. Prof. S. Ranjan, Allahabad
10. Prof. R. Misra, Varanasi
11. Prof. M. D. L. Srivastava, Allahabad
12. Prof. W. D. West, Sagar
13. Dr. S. P. Raychaudhuri, New Delhi
14. Dr. R. N. Tandon, Allahabad (*Secretary*)

CONTENTS

The Mission Press, Allahabad